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PTO/SB/05 (2/98)

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for nonprovisional applications under 37 CFR § 1 53(b))

Attorney Docket No.	210121.419C9
First Inventor or Application Identifier	Tony N. Frudakis
Title	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER
Express Mail Label No.	EL615229895US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

1. [ ] General Authorization Form & Fee Transmittal *(Submit an original and a duplicate for fee processing)*
2. [x] Specification [Total Pages] 131 *(preferred arrangement set forth below)*
   - Descriptive Title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [x] Drawing(s) *(35 USC 113)* [Total Sheets] 25
4. Oath or Declaration [Total Pages]
   a. [ ] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b)
5. [ ] Incorporation By Reference *(useable if box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program *(Appendix)*
7. Nucleotide and Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [x] Computer-Readable Copy
   b. [x] Paper Copy (identical to computer copy)
   c. [x] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [x] Return Receipt Postcard
14. [ ] Small Entity Statement(s) [ ] Statement filed in prior application, Status still proper and desired
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [x] Other: Certificate of Express Mail

17. **If a CONTINUING APPLICATION,** *check appropriate box and supply the requisite information below and in a preliminary amendment*

[ ] Continuation [ ] Divisional [x] Continuation-In-Part (CIP) of prior Application No.: 09/577,505

Prior application information: Examiner not assigned Group / Art Unit not assigned

[ ] Claims the benefit of Provisional Application No. _____

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SIGNATURE Jane E. R. Potter Date June 8, 2000

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Filed	:	June 8, 2000
For	:	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER

Docket No. : 210121.419C9

Date : June 8, 2000

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Respectfully submitted,

Seed Intellectual Property Law Group PLLC

Amber Straub/Jeanette West/Susan Johnson

JEP:sds
Enclosures:

Postcard
Form PTO/SB/05
Specification, Claims, Abstract (131 pages)
25 Sheets of Drawings (Figures 1-24)
Sequence Listing (116)
Declaration for Sequence Listing
Diskette for Sequence Listing

COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Patent Application No. 09/577,505, filed May 24, 2000, which is a continuation-in-part of U.S. Patent Application No. 09/534,825, filed March 22, 2000, which is a continuation-in-part of U.S. Patent Application No. 09/429,755, filed October 28, 1999, which is a continuation-in-part of U.S. Patent Application No. 09/289,198, filed April 9, 1999, which is a continuation-in-part of U.S. Patent Application No. 09/062,451, filed April 17, 1998, which is a continuation in part of U.S. Patent Application No. 08/991,789, filed December 11, 1997, which is a continuation-in-part of U.S. Patent Application No. 08/838,762, filed April 9, 1997, which claims priority from International Patent Application No. PCT/US97/00485, filed January 10, 1997, and is a continuation-in-part of U.S. Patent Application No. 08/700,014, filed August 20, 1996, which is a continuation-in-part of U.S. Patent Application No. 08/585,392, filed January 11, 1996, now abandoned.

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to therapy and diagnosis of cancer, such as breast cancer. The invention is more specifically related to polypeptides comprising at least a portion of a breast tumor protein, and to polynucleotides encoding such polypeptides. Such polypeptides and polynucleotides may be used in compositions for prevention and treatment of breast cancer, and for the diagnosis and monitoring of such cancers.

BACKGROUND OF THE INVENTION

Breast cancer is a significant health problem for women in the United States and throughout the world. Although advances have been made in detection and treatment of the disease, breast cancer remains the second leading cause of cancer-related deaths in

women, affecting more than 180,000 women in the United States each year. For women in North America, the life-time odds of getting breast cancer are now one in eight.

No vaccine or other universally successful method for the prevention or treatment of breast cancer is currently available. Management of the disease currently relies on a combination of early diagnosis (through routine breast screening procedures) and aggressive treatment, which may include one or more of a variety of treatments such as surgery, radiotherapy, chemotherapy and hormone therapy. The course of treatment for a particular breast cancer is often selected based on a variety of prognostic parameters, including an analysis of specific tumor markers. *See, e.g.*, Porter-Jordan and Lippman, *Breast Cancer 8*:73-100 (1994). However, the use of established markers often leads to a result that is difficult to interpret, and the high mortality observed in breast cancer patients indicates that improvements are needed in the treatment, diagnosis and prevention of the disease.

Accordingly, there is a need in the art for improved methods for therapy and diagnosis of breast cancer. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for the diagnosis and therapy of cancer, such as breast cancer. In one aspect, the present invention provides polypeptides comprising at least a portion of a breast tumor protein, or a variant thereof. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises a sequence that is encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317; (b) variants of a sequence recited in SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317; and (c) complements of a sequence of (a) or (b). In specific embodiments, the polypeptides of the present invention comprise at least a portion of a tumor protein that includes an

amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 299, 300, 304-306, 308-312 and 314, and variants thereof.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a breast tumor protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

Within other aspects, the present invention provides pharmaceutical compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, immunogenic compositions, or vaccines for prophylactic or therapeutic use are provided. Such compositions comprise a polypeptide or polynucleotide as described above and an immunostimulant.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a breast tumor protein; and (b) a physiologically acceptable carrier.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts and B cells.

Within related aspects, immunogenic compositions, or vaccines, are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, in other aspects, fusion proteins that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins.

Within related aspects, pharmaceutical compositions comprising a fusion protein, or a polynucleotide encoding a fusion protein, in combination with a physiologically acceptable carrier are provided.

Compositions are further provided, within other aspects, that comprise a fusion protein, or a polynucleotide encoding a fusion protein, in combination with an immunostimulant.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a composition as recited above. The patient may be afflicted with breast cancer, in which case the methods provide treatment for the disease, or patient considered at risk for such a disease may be treated prophylactically.

The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a breast tumor protein, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating $CD4^+$ and/or $CD8^+$ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at

least an immunogenic portion of a breast tumor protein; (ii) a polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expressed such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

Within further aspects, the present invention provides methods for determining the presence or absence of a cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody. The cancer may be breast cancer.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of

mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein; (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the differential display PCR products, separated by gel electrophoresis, obtained from cDNA prepared from normal breast tissue (lanes 1 and 2) and from cDNA prepared from breast tumor tissue from the same patient (lanes 3 and 4). The arrow indicates the band corresponding to B18Ag1.

Figure 2 is a northern blot comparing the level of B18Ag1 mRNA in breast tumor tissue (lane 1) with the level in normal breast tissue.

Figure 3 shows the level of B18Ag1 mRNA in breast tumor tissue compared to that in various normal and non-breast tumor tissues as determined by RNase protection assays.

Figure 4 is a genomic clone map showing the location of additional retroviral sequences obtained from ends of XbaI restriction digests (provided in SEQ ID NO:3 - SEQ ID NO:10) relative to B18Ag1.

Figures 5A and 5B show the sequencing strategy, genomic organization and predicted open reading frame for the retroviral element containing B18Ag1.

Figure 6 shows the nucleotide sequence of the representative breast tumor-specific cDNA B18Ag1.

Figure 7 shows the nucleotide sequence of the representative breast tumor-specific cDNA B17Ag1.

Figure 8 shows the nucleotide sequence of the representative breast tumor-specific cDNA B17Ag2.

Figure 9 shows the nucleotide sequence of the representative breast tumor-specific cDNA B13Ag2a.

Figure 10 shows the nucleotide sequence of the representative breast tumor-specific cDNA B13Ag1b.

Figure 11 shows the nucleotide sequence of the representative breast tumor-specific cDNA B13Ag1a.

Figure 12 shows the nucleotide sequence of the representative breast tumor-specific cDNA B11Ag1.

Figure 13 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA3c.

Figure 14 shows the nucleotide sequence of the representative breast tumor-specific cDNA B9CG1.

Figure 15 shows the nucleotide sequence of the representative breast tumor-specific cDNA B9CG3.

Figure 16 shows the nucleotide sequence of the representative breast tumor-specific cDNA B2CA2.

Figure 17 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA1.

Figure 18 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA2.

Figure 19 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA3.

Figure 20 shows the nucleotide sequence of the representative breast tumor-specific cDNA B4CA1.

Figure 21A depicts RT-PCR analysis of breast tumor genes in breast tumor tissues (lanes 1-8) and normal breast tissues (lanes 9-13) and H_2O (lane 14).

Figure 21B depicts RT-PCR analysis of breast tumor genes in prostate tumors (lane 1, 2), colon tumors (lane 3), lung tumor (lane 4), normal prostate (lane 5), normal colon (lane 6), normal kidney (lane 7), normal liver (lane 8), normal lung (lane 9), normal ovary (lanes 10, 18), normal pancreases (lanes 11, 12), normal skeletal muscle (lane 13), normal skin (lane 14), normal stomach (lane 15), normal testes (lane 16), normal small intestine (lane 17), HBL-100 (lane 19), MCF-12A (lane 20), breast tumors (lanes 21-23), H_2O (lane 24), and colon tumor (lane 25).

Figure 22 shows the recognition of a B11Ag1 peptide (referred to as B11-8) by an anti-B11-8 CTL line.

Figure 23 shows the recognition of a cell line transduced with the antigen B11Ag1 by the B11-8 specific clone A1.

Figure 24 shows recognition of a lung adenocarcinoma line (LT-140-22) and a breast adenocarcinoma line (CAMA-1) by the B11-8 specific clone A1.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for using the compositions, for example in the therapy and diagnosis of cancer, such as breast cancer. Certain illustrative compositions described herein include breast tumor polypeptides, polynucleotides encoding such polypeptides, binding agents such as antibodies, antigen presenting cells (APCs) and/or immune system cells (*e.g.,* T cells). A "breast tumor protein," as the term is used herein, refers generally to a protein that is expressed in breast tumor cells at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in a normal tissue, as determined using a representative assay provided herein. Certain breast tumor proteins are tumor proteins that react detectably (within an immunoassay, such as an ELISA or Western blot) with antisera of a patient afflicted with breast cancer.

Therefore, in accordance with the above, and as described further below, the present invention provides illustrative polynucleotide compositions having sequences set forth in SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317, illustrative polypeptide compositions having amino acid sequences set forth in SEQ ID NO: 299, 300, 304-306, 308-312 and 314, antibody compositions capable of binding such polypeptides, and numerous additional embodiments employing such compositions, for example in the detection, diagnosis and/or therapy of human breast cancer.

POLYNUCLEOTIDE COMPOSITIONS

As used herein, the terms "DNA segment" and "polynucleotide" refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a polypeptide refers to a DNA segment that contains one or more coding sequences yet is substantially isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the terms "DNA segment" and "polynucleotide" are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

As will be understood by those skilled in the art, the DNA segments of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

"Isolated," as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA segment does not contain large portions of unrelated coding DNA, such as large chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.,* an endogenous sequence that encodes a breast tumor protein or a portion thereof) or may comprise a variant, or a biological or antigenic functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. The term "variants" also encompasses homologous genes of xenogenic origin.

When comparing polynucleotide or polypeptide sequences, two sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons

between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS 5*:151-153; Myers, E.W. and Muller W. (1988) *CABIOS 4*:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol. 4*:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA 80*:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0

algorithms, which are described in Altschul *et al.* (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.,* gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.,* the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Therefore, the present invention encompasses polynucleotide and polypeptide sequences having substantial identity to the sequences disclosed herein, for example those comprising at least 50% sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (*e.g.*, BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides and polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like,

(including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

In other embodiments, the present invention is directed to polynucleotides that are capable of hybridizing under moderately stringent conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

PROBES AND PRIMERS

In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization. As such, it is contemplated that nucleic acid segments that comprise a sequence region of at least about 15 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 15 nucleotide long contiguous sequence disclosed

herein will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to a sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned, such as the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence disclosed herein, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequence set forth in SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317, or to any continuous portion of the sequence, from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors. For example, one may wish to employ primers from towards the termini of the total sequence.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S. Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

The nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of the entire gene or gene fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, less stringent (reduced stringency) hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ salt

conditions such as those of from about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

POLYNUCLEOTIDE IDENTIFICATION AND CHARACTERIZATION

Polynucleotides may be identified, prepared and/or manipulated using any of a variety of well established techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.,* expression that is at least two fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena *et al.*, *Proc. Natl. Acad. Sci. USA 93*:10614-10619, 1996 and Heller *et al.*, *Proc. Natl. Acad. Sci. USA 94*:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as breast tumor cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (*e.g.,* a breast tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries

may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia *et al.*, *Nucl. Acids Res. 16*:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by

amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom *et al.*, *PCR Methods Applic. 1*:111-19, 1991) and walking PCR (Parker *et al.*, *Nucl. Acids. Res. 19*:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

POLYNUCLEOTIDE EXPRESSION IN HOST CELLS

In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or

eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. *et al.* (1995) *Science 269*:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, T. (1983) Proteins, Structures

and Molecular Principles, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. *et al.* (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. *et al.* (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the

vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of .beta.-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) *J. Biol. Chem. 264*:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel *et al.* (supra) and Grant *et al.* (1987) *Methods Enzymol.* 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J. 6*:307-311. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. *et al.* (1984) *EMBO J. 3*:1671-1680; Broglie, R. *et al.* (1984) *Science 224*:838-843; and Winter, J. *et al.* (1991) *Results Probl. Cell Differ. 17*:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which the polypeptide of interest may be expressed (Engelhard, E. K. *et al.* (1994) *Proc. Natl. Acad. Sci. 91* :3224-3227).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci. 81*:3655-3659). In addition,

transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. *et al.* (1994) *Results Probl. Cell Differ. 20*:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation. glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells

may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) *Cell 11*:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1990) *Cell 22*:817-23) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. *et al.* (1980) *Proc. Natl. Acad. Sci. 77*:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. et al (1981) *J. Mol. Biol. 150*:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci. 85*:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. *et al.* (1995) *Methods Mol. Biol. 55*:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene

in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. *et al.* (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul. Minn.) and Maddox, D. E. *et al.* (1983; *J. Exp. Med. 158*:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen. San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. *et al.* (1992, *Prot. Exp. Purif. 3*:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. *et al.* (1993; *DNA Cell Biol. 12*:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc. 85*:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin

Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

SITE-SPECIFIC MUTAGENESIS

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent polypeptides, through specific mutagenesis of the underlying polynucleotides that encode them. The technique, well-known to those of skill in the art, further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the antigenicity of a polypeptide vaccine. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and

double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as

amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

POLYNUCLEOTIDE AMPLIFICATION TECHNIQUES

A number of template dependent processes are available to amplify the target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCR™, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (*e.g.*, *Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCR™ amplification procedure may be performed in order to quantify the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Another method for amplification is the ligase chain reaction (referred to as LCR), disclosed in Eur. Pat. Appl. Publ. No. 320,308 (specifically incorporated herein by

reference in its entirety). In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR™, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Patent No. 4,883,750, incorporated herein by reference in its entirety, describes an alternative method of amplification similar to LCR for binding probe pairs to a target sequence.

Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880, incorporated herein by reference in its entirety, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence that can then be detected.

An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[α-thio]triphosphates in one strand of a restriction site (Walker *et al.*, 1992, incorporated herein by reference in its entirety), may also be useful in the amplification of nucleic acids in the present invention.

Strand Displacement Amplification (SDA) is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.* nick translation. A similar method, called Repair Chain Reaction (RCR) is another method of amplification which may be useful in the present invention and is involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA.

Sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having a 3' and 5' sequences of non-target DNA and an internal or "middle"

sequence of the target protein specific RNA is hybridized to DNA which is present in a sample. Upon hybridization, the reaction is treated with RNaseH, and the products of the probe are identified as distinctive products by generating a signal that is released after digestion. The original template is annealed to another cycling probe and the reaction is repeated. Thus, CPR involves amplifying a signal generated by hybridization of a probe to a target gene specific expressed nucleic acid.

Still other amplification methods described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety, may be used in accordance with the present invention. In the former application, "modified" primers are used in a PCR-like, template and enzyme dependent synthesis. The primers may be modified by labeling with a capture moiety (*e.g.,* biotin) and/or a detector moiety (*e.g.,* enzyme). In the latter application, an excess of labeled probes is added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the target sequence is released intact to be bound by excess probe. Cleavage of the labeled probe signals the presence of the target sequence.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh *et al.,* 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer that has sequences specific to the target sequence. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat-denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse

transcribed into DNA, and transcribed once again with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target-specific sequences.

Eur. Pat. Appl. Publ. No. 329,822, incorporated herein by reference in its entirety, disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a first template for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from resulting DNA:RNA duplex by the action of ribonuclease H (RNase H, an RNase specific for RNA in a duplex with either DNA or RNA). The resultant ssDNA is a second template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to its template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow" fragment of *E. coli* DNA polymerase I), resulting as a double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA.

PCT Intl. Pat. Appl. Publ. No. WO 89/06700, incorporated herein by reference in its entirety, disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic; *i.e.* new templates are not produced from the resultant RNA transcripts. Other amplification methods include "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) which are well-known to those of skill in the art.

Methods based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby

amplifying the di-oligonucleotide (Wu and Dean, 1996, incorporated herein by reference in its entirety), may also be used in the amplification of DNA sequences of the present invention.

BIOLOGICAL FUNCTIONAL EQUIVALENTS

Modification and changes may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a polypeptide with desirable characteristics. As mentioned above, it is often desirable to introduce one or more mutations into a specific polynucleotide sequence. In certain circumstances, the resulting encoded polypeptide sequence is altered by this mutation, or in other cases, the sequence of the polypeptide is unchanged by one or more mutations in the encoding polynucleotide.

When it is desirable to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, second-generation molecule, the amino acid changes may be achieved by changing one or more of the codons of the encoding DNA sequence, according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

TABLE 1

Amino Acids			Codons					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been

assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (–0.4); threonine (–0.7); serine (–0.8); tryptophan (–0.9); tyrosine (–1.3); proline (–1.6); histidine (–3.2); glutamate (–3.5); glutamine (–3.5); aspartate (–3.5); asparagine (–3.5); lysine (–3.9); and arginine (–4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5); leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their

hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

IN VIVO POLYNUCLEOTIDE DELIVERY TECHNIQUES

In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety or well known approaches, several of which are outlined below for the purpose of illustration.

1. ADENOVIRUS

One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus and Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because

adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5′-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is

dispensable from the adenovirus genome (Jones and Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham and Prevec, 1991). In nature, adenovirus can package approximately 105% of the wild-type genome (Ghosh-Choudhury *et al.*, 1987), providing capacity for about 2 extra kB of DNA. Combined with the approximately 5.5 kB of DNA that is replaceable in the E1 and E3 regions, the maximum capacity of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, *e.g.*, Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the currently preferred helper cell line is 293.

Recently, Racher *et al.* (1995) disclosed improved methods for culturing 293 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, *e.g.*, 10^9-10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch *et al.*, 1963; Top *et al.*, 1971), demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors.

Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus and Horwitz, 1992; Graham and Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet and Perricaudet, 1991; Stratford-Perricaudet *et al.*, 1990; Rich *et al.*, 1993). Studies in administering recombinant

adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.*, 1992), muscle injection (Ragot *et al.*, 1993), peripheral intravenous injections (Herz and Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993).

2. RETROVIRUSES

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5′ and 3′ ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding one or more oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene

transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

3. ADENO-ASSOCIATED VIRUSES

AAV (Ridgeway, 1988; Hermonat and Muzycska, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replications is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka and McLaughlin, 1988).

The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs. There are two major genes in the AAV genome: *rep* and *cap*. The *rep* gene codes for proteins responsible for viral replications, whereas *cap* codes for capsid protein VP1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential *cis* components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral

promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of rep proteins, and transcription from p40 produces the capsid proteins (Hermonat and Muzyczka, 1984).

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering the antisense constructs of the present invention.

AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilize rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

4. OTHER VIRAL VECTORS AS EXPRESSION CONSTRUCTS

Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar *et al.*, 1988), lentiviruses, polio viruses and herpes viruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar *et al.*, 1988; Horwich *et al.*, 1990).

With the recent recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich *et al.*, 1990). This suggested that large portions of the genome could be replaced with foreign genetic material. The hepatotropism and persistence (integration) were particularly attractive

properties for liver-directed gene transfer. Chang *et al.* (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media containing high titers of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang *et al.*, 1991).

5. NON-VIRAL VECTORS

In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is *via* viral infection where the expression construct is encapsulated in an infectious viral particle.

Once the expression construct has been delivered into the cell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell

membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky *et al.* (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner *in vivo* and express the gene product.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded *in vivo* (Yang *et al.*, 1990; Zelenin *et al.*, 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, *i.e. ex vivo* treatment. Again, DNA encoding a particular gene may be delivered *via* this method and still be incorporated by the present invention.

ANTISENSE OLIGONUCLEOTIDES

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for

polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

The targeting of antisense oligonucleotides to mRNA is thus one mechanism to shut down protein synthesis, and, consequently, represents a powerful and targeted therapeutic approach. For example, the synthesis of polygalactauronase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829, each specifically incorporated herein by reference in its entirety). Further, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal $GABA_A$ receptor and human EGF (Jaskulski *et al.*, 1988; Vasanthakumar and Ahmed, 1989; Peris *et al.*, 1998; U. S. Patent 5,801,154; U. S. Patent 5,789,573; U. S. Patent 5,718,709 and U. S. Patent 5,610,288, each specifically incorporated herein by reference in its entirety). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, *e.g.* cancer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683, each specifically incorporated herein by reference in its entirety).

Therefore, in exemplary embodiments, the invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonucleotides comprise RNA or derivatives thereof. In a third embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In a fourth embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, preferred compositions comprise a sequence region that is complementary, and more preferably substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides disclosed herein.

Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence (*i.e.* in these illustrative examples the rat and human sequences) and determination of secondary structure, T_m, binding energy, relative stability, and antisense compositions were selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell.

Highly preferred target regions of the mRNA, are those which are at or near the AUG translation initiation codon, and those sequences which were substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations were performed using v.4 of the OLIGO primer analysis software (Rychlik, 1997) and the BLASTN 2.0.5 algorithm software (Altschul *et al.,* 1997).

The use of an antisense delivery method employing a short peptide vector, termed MPG (27 residues), is also contemplated. The MPG peptide contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain from the nuclear localization sequence of SV40 T-antigen (Morris *et al.*, 1997). It has been demonstrated that several molecules of the MPG peptide coat the antisense oligonucleotides and can be delivered into cultured mammalian cells in less than 1 hour with relatively high efficiency (90%). Further, the interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and the ability to cross the plasma membrane (Morris *et al.*, 1997).

RIBOZYMES

Although proteins traditionally have been used for catalysis of nucleic acids, another class of macromolecules has emerged as useful in this endeavor. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cech, 1987; Gerlach *et al.*, 1987; Forster and Symons, 1987). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et*

al., 1981; Michel and Westhof, 1990; Reinhold-Hurek and Shub, 1992). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, 1989; Cech *et al.*, 1981). For example, U. S. Patent No. 5,354,855 (specifically incorporated herein by reference) reports that certain ribozymes can act as endonucleases with a sequence specificity greater than that of known ribonucleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon *et al.*, 1991; Sarver *et al.*, 1990). Recently, it was reported that ribozymes elicited genetic changes in some cells lines to which they were applied; the altered genes included the oncogenes H-*ras*, c-*fos* and genes of HIV. Most of this work involved the modification of a target mRNA, based on a specific mutant codon that is cleaved by a specific ribozyme.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to

a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, 1992). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are described by Rossi *et al.* (1992). Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz (1989), Hampel *et al.* (1990) and U. S. Patent 5,631,359 (specifically incorporated herein by reference). An example of the hepatitis δ virus motif is described by Perrotta and Been (1992); an example of the RNaseP motif is described by Guerrier-Takada *et al.* (1983); Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990; Saville and Collins, 1991; Collins and Olive, 1993); and an example of the Group I intron is described in (U. S. Patent 4,987,071, specifically incorporated herein by reference). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

In certain embodiments, it may be important to produce enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target, such as

one of the sequences disclosed herein. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNA. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) may also be used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. Alternatively, catalytic RNA molecules can be expressed within cells from eukaryotic promoters (*e.g.*, Scanlon *et al.*, 1991; Kashani-Sabet *et al.*, 1992; Dropulic *et al.*, 1992; Weerasinghe *et al.*, 1991; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Sarver *et al.*, 1990). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Int. Pat. Appl. Publ. No. WO 93/23569, and Int. Pat. Appl. Publ. No. WO 94/02595, both hereby incorporated by reference; Ohkawa *et al.*, 1992; Taira *et al.*, 1991; and Ventura *et al.*, 1993).

Ribozymes may be added directly, or can be complexed with cationic lipids, lipid complexes, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as described. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Hammerhead or hairpin ribozymes may be individually analyzed by computer folding (Jaeger *et al.*, 1989) to assess whether the ribozyme sequences fold into

the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 or so bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Ribozymes of the hammerhead or hairpin motif may be designed to anneal to various sites in the mRNA message, and can be chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.* (1987) and in Scaringe *et al.* (1990) and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5′-end, and phosphoramidites at the 3′-end. Average stepwise coupling yields are typically >98%. Hairpin ribozymes may be synthesized in two parts and annealed to reconstruct an active ribozyme (Chowrira and Burke, 1992). Ribozymes may be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2′-amino, 2′-C-allyl, 2′-flouro, 2′-o-methyl, 2′-H (for a review see *e.g.,* Usman and Cedergren, 1992). Ribozymes may be purified by gel electrophoresis using general methods or by high pressure liquid chromatography and resuspended in water.

Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Perrault *et al*, 1990; Pieken *et al.*, 1991; Usman and Cedergren, 1992; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U. S. Patent 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan *et al.* (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be

administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Int. Pat. Appl. Publ. No. WO 94/02595 and Int. Pat. Appl. Publ. No. WO 93/23569, each specifically incorporated herein by reference.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, *etc.*) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990; Gao and Huang, 1993; Lieber *et al.*, 1993; Zhou *et al.*, 1990). Ribozymes expressed from such promoters can function in mammalian cells (*e.g.* Kashani-Saber *et al.*, 1992; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Yu *et al.*, 1993; L'Huillier *et al.*, 1992; Lisziewicz *et al.*, 1993). Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

Ribozymes may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. They can also be used to assess levels of the target RNA molecule. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These studies will lead to better treatment of the disease progression by affording the possibility of combinational therapies (*e.g.*, multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes are well known in the art, and include detection of the presence of mRNA associated with an IL-5 related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

PEPTIDE NUCLEIC ACIDS

In certain embodiments, the inventors contemplate the use of peptide nucleic acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone (Good and Nielsen, 1997). PNA is able to be utilized in a number methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (1997) and is incorporated herein by reference. As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and such PNA compositions may be used to regulate, alter,

decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, 1991; Hanvey *et al.*, 1992; Hyrup and Nielsen, 1996; Neilsen, 1996). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc (Dueholm *et al.*, 1994) or Fmoc (Thomson *et al.*, 1995) protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used (Christensen *et al.,* 1995).

PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoc protocols are straightforward using manual or automated protocols (Norton *et al.*, 1995). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjacent purines, one should repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography (Norton *et al.*, 1995) providing yields and purity of product similar to those observed during the synthesis of peptides.

Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be modified after synthesis by coupling to an introduced lysine or cysteine. The ease with which PNAs can be modified facilitates optimization for better solubility or for specific

functional requirements. Once synthesized, the identity of PNAs and their derivatives can be confirmed by mass spectrometry. Several studies have made and utilized modifications of PNAs (Norton *et al.*, 1995; Haaima *et al.*, 1996; Stetsenko *et al.*, 1996; Petersen *et al.*, 1995; Ulmann *et al.*, 1996; Koch *et al.*, 1995; Orum *et al.*, 1995; Footer *et al.*, 1996; Griffith *et al.*, 1995; Kremsky *et al.*, 1996; Pardridge *et al.*, 1995; Boffa *et al.*, 1995; Landsdorp *et al.*, 1996; Gambacorti-Passerini *et al.*, 1996; Armitage *et al.*, 1997; Seeger *et al.*, 1997; Ruskowski *et al.*, 1997). U.S. Patent No. 5,700,922 discusses PNA-DNA-PNA chimeric molecules and their uses in diagnostics, modulating protein in organisms, and treatment of conditions susceptible to therapeutics.

In contrast to DNA and RNA, which contain negatively charged linkages, the PNA backbone is neutral. In spite of this dramatic alteration, PNAs recognize complementary DNA and RNA by Watson-Crick pairing (Egholm *et al.*, 1993), validating the initial modeling by Nielsen *et al.* (1991). PNAs lack 3′ to 5′ polarity and can bind in either parallel or antiparallel fashion, with the antiparallel mode being preferred (Egholm *et al.*, 1993).

Hybridization of DNA oligonucleotides to DNA and RNA is destabilized by electrostatic repulsion between the negatively charged phosphate backbones of the complementary strands. By contrast, the absence of charge repulsion in PNA-DNA or PNA-RNA duplexes increases the melting temperature (T_m) and reduces the dependence of T_m on the concentration of mono- or divalent cations (Nielsen *et al.*, 1991). The enhanced rate and affinity of hybridization are significant because they are responsible for the surprising ability of PNAs to perform strand invasion of complementary sequences within relaxed double-stranded DNA. In addition, the efficient hybridization at inverted repeats suggests that PNAs can recognize secondary structure effectively within double-stranded DNA. Enhanced recognition also occurs with PNAs immobilized on surfaces, and Wang *et al.* have shown that support-bound PNAs can be used to detect hybridization events (Wang *et al.*, 1996).

One might expect that tight binding of PNAs to complementary sequences would also increase binding to similar (but not identical) sequences, reducing the sequence

specificity of PNA recognition. As with DNA hybridization, however, selective recognition can be achieved by balancing oligomer length and incubation temperature. Moreover, selective hybridization of PNAs is encouraged by PNA-DNA hybridization being less tolerant of base mismatches than DNA-DNA hybridization. For example, a single mismatch within a 16 bp PNA-DNA duplex can reduce the T_m by up to 15°C (Egholm *et al.*, 1993). This high level of discrimination has allowed the development of several PNA-based strategies for the analysis of point mutations (Wang *et al.*, 1996; Carlsson *et al.*, 1996; Thiede *et al.*, 1996; Webb and Hurskainen, 1996; Perry-O'Keefe *et al.*, 1996).

High-affinity binding provides clear advantages for molecular recognition and the development of new applications for PNAs. For example, 11-13 nucleotide PNAs inhibit the activity of telomerase, a ribonucleo-protein that extends telomere ends using an essential RNA template, while the analogous DNA oligomers do not (Norton *et al.*, 1996).

Neutral PNAs are more hydrophobic than analogous DNA oligomers, and this can lead to difficulty solubilizing them at neutral pH, especially if the PNAs have a high purine content or if they have the potential to form secondary structures. Their solubility can be enhanced by attaching one or more positive charges to the PNA termini (Nielsen *et al.*, 1991).

Findings by Allfrey and colleagues suggest that strand invasion will occur spontaneously at sequences within chromosomal DNA (Boffa *et al.*, 1995; Boffa *et al.*, 1996). These studies targeted PNAs to triplet repeats of the nucleotides CAG and used this recognition to purify transcriptionally active DNA (Boffa *et al.*, 1995) and to inhibit transcription (Boffa *et al.*, 1996). This result suggests that if PNAs can be delivered within cells then they will have the potential to be general sequence-specific regulators of gene expression. Studies and reviews concerning the use of PNAs as antisense and anti-gene agents include Nielsen *et al.* (1993b), Hanvey *et al.* (1992), and Good and Nielsen (1997). Koppelhus *et al.* (1997) have used PNAs to inhibit HIV-1 inverse transcription, showing that PNAs may be used for antiviral therapies.

Methods of characterizing the antisense binding properties of PNAs are discussed in Rose (1993) and Jensen *et al.* (1997). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

Other applications of PNAs include use in DNA strand invasion (Nielsen *et al.,* 1991), antisense inhibition (Hanvey *et al.*, 1992), mutational analysis (Orum *et al.*, 1993), enhancers of transcription (Mollegaard *et al.*, 1994), nucleic acid purification (Orum *et al.*, 1995), isolation of transcriptionally active genes (Boffa *et al.*, 1995), blocking of transcription factor binding (Vickers *et al.*, 1995), genome cleavage (Veselkov *et al.*, 1996), biosensors (Wang *et al.*, 1996), *in situ* hybridization (Thisted *et al.*, 1996), and in a alternative to Southern blotting (Perry-O'Keefe, 1996).

POLYPEPTIDE COMPOSITIONS

The present invention, in other aspects, provides polypeptide compositions. Generally, a polypeptide of the invention will be an isolated polypeptide (or an epitope, variant, or active fragment thereof) derived from a mammalian species. Preferably, the polypeptide is encoded by a polynucleotide sequence disclosed herein or a sequence which hybridizes under moderately stringent conditions to a polynucleotide sequence disclosed herein. Alternatively, the polypeptide may be defined as a polypeptide which comprises a contiguous amino acid sequence from an amino acid sequence disclosed herein, or which polypeptide comprises an entire amino acid sequence disclosed herein.

In the present invention, a polypeptide composition is also understood to comprise one or more polypeptides that are immunologically reactive with antibodies generated against a polypeptide of the invention, particularly a polypeptide having the amino acid sequence disclosed in SEQ ID NO: 299, 300, 304-306, 308-312 and 314, or to active fragments, or to variants or biological functional equivalents thereof.

Likewise, a polypeptide composition of the present invention is understood to comprise one or more polypeptides that are capable of eliciting antibodies that are

immunologically reactive with one or more polypeptides encoded by one or more contiguous nucleic acid sequences contained in SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317, or to active fragments, or to variants thereof, or to one or more nucleic acid sequences which hybridize to one or more of these sequences under conditions of moderate to high stringency. Particularly illustrative polypeptides include the amino acid sequence disclosed in SEQ ID NO: 299, 300, 304-306, 308-312 and 314.

As used herein, an active fragment of a polypeptide includes a whole or a portion of a polypeptide which is modified by conventional techniques, *e.g.*, mutagenesis, or by addition, deletion, or substitution, but which active fragment exhibits substantially the same structure function, antigenicity, etc., as a polypeptide as described herein.

In certain illustrative embodiments, the polypeptides of the invention will comprise at least an immunogenic portion of a breast tumor protein or a variant thereof, as described herein. As noted above, a "breast tumor protein" is a protein that is expressed by breast tumor cells. Proteins that are breast tumor proteins also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with breast cancer. Polypeptides as described herein may be of any length. Additional sequences derived from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

An "immunogenic portion," as used herein is a portion of a protein that is recognized (*i.e.,* specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of a breast tumor protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.,* 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247

(Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.,* they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native breast tumor protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (*e.g.,* in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I-labeled Protein A.

As noted above, a composition may comprise a variant of a native breast tumor protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native breast tumor protein in one or more substitutions, deletions, additions and/or insertions, such that the immunogenicity of the polypeptide is not substantially diminished. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (*e.g.,* 1-30

amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

Polypeptide variants encompassed by the present invention include those exhibiting at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described above) to the polypeptides disclosed herein.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-

His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological

fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene 40*:39-46, 1985; Murphy *et al.*, *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may

generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided. Such proteins comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute *et al. New Engl. J. Med.*, *336*:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-

terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

BINDING AGENTS

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a breast tumor protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a breast tumor protein if it reacts at a detectable level (within, for example, an ELISA) with a breast tumor protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex

formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a cancer, such as breast cancer, using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a breast tumor protein will generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.,* blood, sera, sputum, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.,* mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as

bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* *6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y, ^{123}I, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter *et al.*), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn *et al.*), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler *et al.*).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato *et al.*), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih *et al.*). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide

agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison *et al.* discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a breast tumor protein. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a breast tumor polypeptide, polynucleotide encoding a breast tumor polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, a breast tumor polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a breast tumor polypeptide if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* *54*:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (*e.g.*, by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a breast tumor polypeptide (100 ng/ml - 100 μg/ml, preferably 200 ng/ml - 25 μg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (*e.g.*, TNF or IFN-γ) is indicative of T cell activation (*see* Coligan *et al.*, Current Protocols in Immunology, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a breast tumor polypeptide, polynucleotide or polypeptide-expressing APC may be $CD4^+$ and/or $CD8^+$. Breast tumor protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, $CD4^+$ or $CD8^+$ T cells that proliferate in response to a breast tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a breast tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a breast tumor polypeptide. Alternatively, one or more T cells that

proliferate in the presence of a breast tumor protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell and/or antibody compositions disclosed herein in pharmaceutically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will also be understood that, if desired, the nucleic acid segment, RNA, DNA or PNA compositions that express a polypeptide as disclosed herein may be administered in combination with other agents as well, such as, *e.g.*, other proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.,* oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

1. ORAL DELIVERY

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be

enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz *et al.*, 1997; Hwang *et al.*, 1998; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in

the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

2. INJECTABLE DELIVERY

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally as described in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U. S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture

and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally,

dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable

for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

3. NASAL DELIVERY

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described *e.g.,* in U. S. Patent 5,756,353 and U. S. Patent 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U. S. Patent 5,780,045 (specifically incorporated herein by reference in its entirety).

4. LIPOSOME-, NANOCAPSULE-, AND MICROPARTICLE-MEDIATED DELIVERY

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the nucleic acids or constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon and Papahadjopoulos, 1988; Allen and

Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, 1990; Muller *et al.*, 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath and Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta and Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller and Baltimore, 1984), transcription factors and allosteric effectors (Nicolau and Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trails examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b; Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori and Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, *i.e.* in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-

bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977; 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the

bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for h or days, depending on their composition, and half lives in the blood range from min to several h. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific antigenic receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface

components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be are easily made, as described (Couvreur *et al.*, 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

IMMUNOGENIC COMPOSITIONS

In certain preferred embodiments of the present invention, immunogenic compositions, or vaccines, are provided. The immunogenic compositions will generally comprise one or more pharmaceutical compositions, such as those discussed above, in combination with an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (*e.g.,* polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.,* Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and immunogenic compositions within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For

example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition.

Illustrative immunogenic compositions may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems 15*:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch *et al.*, *Proc. Natl. Acad. Sci. USA 86*:317-321, 1989; Flexner *et al.*, *Ann. N.Y. Acad. Sci. 569*:86-103, 1989; Flexner *et al.*, *Vaccine 8*:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques 6*:616-627, 1988; Rosenfeld *et al.*, *Science 252*:431-434, 1991; Kolls *et al.*, *Proc. Natl. Acad. Sci. USA 91*:215-219, 1994; Kass-Eisler *et al.*, *Proc. Natl. Acad. Sci. USA 90*:11498-11502, 1993; Guzman *et al.*, *Circulation 88*:2838-2848, 1993; and Guzman *et al.*, *Cir. Res. 73*:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that an immunogenic composition may comprise both a

polynucleotide and a polypeptide component. Such immunogenic compositions may provide for an enhanced immune response.

It will be apparent that an immunogenic composition may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.,* salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.,* sodium, potassium, lithium, ammonium, calcium and magnesium salts).

While any suitable carrier known to those of ordinary skill in the art may be employed in the compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.,* polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252. One may also employ a carrier comprising the particulate-protein complexes described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

Such compositions may also comprise buffers (*e.g.,* neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.,* glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood

of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the immunogenic compositions of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the immunogenic compositions provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (*e.g.*, IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.*, IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of an immunogenic composition as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol. 7*:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; *see* US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science 273*:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.,* SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties.

Any immunogenic composition provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (*i.e.,* a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound

following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes *et al.*, *Vaccine 14*:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.,* a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.,* U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and immunogenic compositions to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.,* matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature 392*:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med. 50*:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within an immunogenic composition (*see* Zitvogel *et al.*, *Nature Med. 4*:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically

characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.,* CD54 and CD11) and costimulatory molecules (*e.g.,* CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a breast tumor protein (or portion or other variant thereof) such that the breast tumor polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi *et al.*, *Immunology and cell Biology 75*:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the breast tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (*e.g.,* vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (*e.g.,* a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Immunogenic compositions and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a immunogenic composition or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

CANCER THERAPY

In further aspects of the present invention, the compositions described herein may be used for immunotherapy of cancer, such as breast cancer. Within such methods, pharmaceutical compositions and immunogenic compositions are typically administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Accordingly, the above pharmaceutical compositions and immunogenic compositions may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor. Pharmaceutical compositions and immunogenic compositions may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. Administration may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (such as polypeptides and polynucleotides as provided herein).

Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other

vectors or effector cells for adoptive immunotherapy. The polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy.

Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above, immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast and/or B cells, may be pulsed with immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (*see*, for example, Cheever *et al.*, *Immunological Reviews 157*:177, 1997).

Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions

and immunogenic compositions may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e.,* untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such immunogenic compositions should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in treated patients as compared to non-treated patients. In general, for pharmaceutical compositions and immunogenic compositions comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 25 μg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a breast tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

CANCER DETECTION AND DIAGNOSIS

In general, a cancer may be detected in a patient based on the presence of one or more breast tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, sputum urine and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as breast cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, a breast tumor sequence should be present at a level that is at least three fold higher in tumor tissue than in normal tissue

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. *See*, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the

labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length breast tumor proteins and portions thereof to which the binding agent binds, as described above.

The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 μg, and preferably about 100 ng to about 1 μg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group

on the support with an amine and an active hydrogen on the binding partner (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with breast cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, such as breast cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett *et al.*, *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the

false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use breast tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample.

The detection of such breast tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a breast tumor protein in a biological sample. Within certain methods, a biological sample comprising $CD4^+$ and/or $CD8^+$ T cells isolated from a patient is incubated with a breast tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with polypeptide (*e.g.*, 5 - 25 μg/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of breast tumor polypeptide to serve as a control. For $CD4^+$ T cells, activation is preferably detected by evaluating proliferation of the T cells. For $CD8^+$ T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a breast tumor protein in a biological sample. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of a breast tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for (*i.e.,* hybridizes to) a polynucleotide encoding the breast tumor protein. The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding a breast tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a breast tumor protein that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence recited in SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317. Techniques for both PCR based assays and hybridization assays are well known in the art (*see*, for example, Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, *51*:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989).

One preferred assay employs RT-PCR, in which PCR is applied in conjunction with reverse transcription. Typically, RNA is extracted from a biological sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

In another embodiment, the compositions described herein may be used as markers for the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be

performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound binding agent may then be detected directly or indirectly via a reporter group. Such binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple breast tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

DIAGNOSTIC KITS

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a breast tumor protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a breast tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a breast tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a breast tumor protein.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

PREPARATION OF BREAST TUMOR-SPECIFIC cDNAs USING DIFFERENTIAL DISPLAY RT-PCR

This Example illustrates the preparation of cDNA molecules encoding breast tumor-specific polypeptides using a differential display screen.

A. Preparation of B18Ag1 cDNA and Characterization of mRNA Expression

Tissue samples were prepared from breast tumor and normal tissue of a patient with breast cancer that was confirmed by pathology after removal from the patient. Normal RNA and tumor RNA was extracted from the samples and mRNA was isolated and converted into cDNA using a $(dT)_{12}AG$ (SEQ ID NO:130) anchored 3' primer. Differential display PCR was then executed using a randomly chosen primer (CTTCAACCTC) (SEQ ID NO:103). Amplification conditions were standard buffer containing 1.5 mM $MgCl_2$, 20 pmol of primer, 500 pmol dNTP, and 1 unit of *Taq* DNA polymerase (Perkin-Elmer, Branchburg, NJ). Forty cycles of amplification were performed using 94°C denaturation for 30 seconds, 42°C annealing for 1 minute, and 72°C extension for 30 seconds. An RNA fingerprint containing 76 amplified products was obtained. Although the RNA fingerprint of breast tumor tissue was over 98% identical to that of the normal breast tissue, a band was repeatedly observed to be specific to the RNA fingerprint pattern of the tumor. This band was cut out of a silver stained gel, subcloned into the T-vector (Novagen, Madison, WI) and sequenced.

The sequence of the cDNA, referred to as B18Ag1, is provided in SEQ ID NO:1. A database search of GENBANK and EMBL revealed that the B18Ag1 fragment initially cloned is 77% identical to the endogenous human retroviral element S71, which is a truncated retroviral element homologous to the Simian Sarcoma Virus (SSV). S71 contains an incomplete *gag* gene, a portion of the *pol* gene and an LTR-like structure at the 3' terminus (*see* Werner et al., *Virology 174*:225-238 (1990)). B18Ag1 is also 64% identical to SSV in the region corresponding to the P30 (gag) locus. B18Ag1 contains

three separate and incomplete reading frames covering a region which shares considerable homology to a wide variety of gag proteins of retroviruses which infect mammals. In addition, the homology to S71 is not just within the *gag* gene, but spans several kb of sequence including an LTR.

B18Ag1-specific PCR primers were synthesized using computer analysis guidelines. RT-PCR amplification (94°C, 30 seconds; 60°C → 42°C, 30 seconds; 72°C, 30 seconds for 40 cycles) confirmed that B18Ag1 represents an actual mRNA sequence present at relatively high levels in the patient's breast tumor tissue. The primers used in amplification were B18Ag1-1 (CTG CCT GAG CCA CAA ATG) (SEQ ID NO:128) and B18Ag1-4 (CCG GAG GAG GAA GCT AGA GGA ATA) (SEQ ID NO:129) at a 3.5 mM magnesium concentration and a pH of 8.5, and B18Ag1-2 (ATG GCT ATT TTC GGG GCC TGA CA) (SEQ ID NO:126) and B18Ag1-3 (CCG GTA TCT CCT CGT GGG TAT T) (SEQ ID NO:127) at 2 mM magnesium at pH 9.5. The same experiments showed exceedingly low to nonexistent levels of expression in this patient's normal breast tissue (*see* Figure 1). RT-PCR experiments were then used to show that B18Ag1 mRNA is present in nine other breast tumor samples (from Brazilian and American patients) but absent in, or at exceedingly low levels in, the normal breast tissue corresponding to each cancer patient. RT-PCR analysis has also shown that the B18Ag1 transcript is not present in various normal tissues (including lymph node, myocardium and liver) and present at relatively low levels in PBMC and lung tissue. The presence of B18Ag1 mRNA in breast tumor samples, and its absence from normal breast tissue, has been confirmed by Northern blot analysis, as shown in Figure 2.

The differential expression of B18Ag1 in breast tumor tissue was also confirmed by RNase protection assays. Figure 3 shows the level of B18Ag1 mRNA in various tissue types as determined in four different RNase protection assays. Lanes 1-12 represent various normal breast tissue samples, lanes 13-25 represent various breast tumor samples; lanes 26-27 represent normal prostate samples; lanes 28-29 represent prostate tumor samples; lanes 30-32 represent colon tumor samples; lane 33 represents normal aorta; lane 34 represents normal small intestine; lane 35 represents normal skin, lane 36

represents normal lymph node; lane 37 represents normal ovary; lane 38 represents normal liver; lane 39 represents normal skeletal muscle; lane 40 represents a first normal stomach sample, lane 41 represents a second normal stomach sample; lane 42 represents a normal lung; lane 43 represents normal kidney; and lane 44 represents normal pancreas. Interexperimental comparison was facilitated by including a positive control RNA of known β-actin message abundance in each assay and normalizing the results of the different assays with respect to this positive control.

RT-PCR and Southern Blot analysis has shown the B18Ag1 locus to be present in human genomic DNA as a single copy endogenous retroviral element. A genomic clone of approximately 12-18 kb was isolated using the initial B18Ag1 sequence as a probe. Four additional subclones were also isolated by XbaI digestion. Additional retroviral sequences obtained from the ends of the XbaI digests of these clones (located as shown in Figure 4) are shown as SEQ ID NO:3 - SEQ ID NO:10, where SEQ ID NO:3 shows the location of the sequence labeled 10 in Figure 4, SEQ ID NO:4 shows the location of the sequence labeled 11-29, SEQ ID NO:5 shows the location of the sequence labeled 3, SEQ ID NO:6 shows the location of the sequence labeled 6, SEQ ID NO:7 shows the location of the sequence labeled 12, SEQ ID NO:8 shows the location of the sequence labeled 13, SEQ ID NO:9 shows the location of the sequence labeled 14 and SEQ ID NO:10 shows the location of the sequence labeled 11-22.

Subsequent studies demonstrated that the 12-18 kb genomic clone contains a retroviral element of about 7.75 kb, as shown in Figures 5A and 5B. The sequence of this retroviral element is shown in SEQ ID NO:141. The numbered line at the top of Figure 5A represents the sense strand sequence of the retroviral genomic clone. The box below this line shows the position of selected restriction sites. The arrows depict the different overlapping clones used to sequence the retroviral element. The direction of the arrow shows whether the single-pass subclone sequence corresponded to the sense or anti-sense strand. Figure 5B is a schematic diagram of the retroviral element containing B18Ag1 depicting the organization of viral genes within the element. The open boxes correspond to predicted reading frames, starting with a methionine, found throughout the element. Each

of the six likely reading frames is shown, as indicated to the left of the boxes, with frames 1-3 corresponding to those found on the sense strand.

Using the cDNA of SEQ ID NO:1 as a probe, a longer cDNA was obtained (SEQ ID NO:227) which contains minor nucleotide differences (less than 1%) compared to the genomic sequence shown in SEQ ID NO:141.

B. Preparation of cDNA Molecules Encoding Other Breast Tumor-Specific Polypeptides

Normal RNA and tumor RNA was prepared and mRNA was isolated and converted into cDNA using a $(dT)_{12}AG$ anchored 3' primer, as described above. Differential display PCR was then executed using the randomly chosen primers of SEQ ID NOs:87-125. Amplification conditions were as noted above, and bands observed to be specific to the RNA fingerprint pattern of the tumor were cut out of a silver stained gel, subcloned into either the T-vector (Novagen, Madison, WI) or the pCRII vector (Invitrogen, San Diego, CA) and sequenced. The sequences are provided in SEQ ID NO:11 - SEQ ID NO:86. Of the 79 sequences isolated, 67 were found to be novel (SEQ ID NOs:11-26 and 28-77) (*see also* Figures 6-20).

An extended DNA sequence (SEQ ID NO:290) for the antigen B15Ag1 (originally identified partial sequence provided in SEQ ID NO:27) was obtained in further studies. Comparison of the sequence of SEQ ID NO:290 with those in the gene bank as described above, revealed homology to the known human β-A activin gene. Further studies led to the isolation of the full-length cDNA sequence for the antigen B21GT2 (also referred to as B311D; originally identified partial cDNA sequence provided in SEQ ID NOs:56). The full-length sequence is provided in SEQ ID NO:307, with the corresponding amino acid sequence being provided in SEQ ID NO:308. Further studies led to the isolation of a splice variant of B311D. The B311D clone of SEQ ID NO:316 was sequenced and a XhoI/NotI fragment from this clone was gel purified and 32P-cDTP labeled by random priming for use as a probe for further screening to obtain additional B311D gene sequence. Two fractions of a human breast tumor cDNA bacterial library were screened using standard techniques. One of the clones isolated in this manner yielded

additional sequence which includes a poly A+ tail. The determined cDNA sequence of this clone (referred to as B311D_BT1_1A) is provided in SEQ ID NO:317. The sequences of SEQ ID NOs:316 and 317 were found to share identity over a 464 bp region, with the sequences diverging near the poly A+ sequence of SEQ ID NO:317.

Subsequent studies identified an additional 146 sequences (SEQ ID NOs:142-289), of which 115 appeared to be novel (SEQ ID NOs:142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288 and 291). To the best of the inventors' knowledge none of the previously identified sequences have heretofore been shown to be expressed at a greater level in human breast tumor tissue than in normal breast tissue.

In further studies, several different splice forms of the antigen B11Ag1 (also referred to as B305D) were isolated, with each of the various splice forms containing slightly different versions of the B11Ag1 coding frame. Splice junction sequences define individual exons which, in various patterns and arrangements, make up the various splice forms. Primers were designed to examine the expression pattern of each of the exons using RT-PCR as described below. Each exon was found to show the same expression pattern as the original B11Ag1 clone, with expression being breast tumor-, normal prostate- and normal testis-specific. The determined cDNA sequences for the isolated protein coding exons are provided in SEQ ID NOs:292-298, respectively. The predicted amino acid sequences corresponding to the sequences of SEQ ID NOs:292 and 298 are provided in SEQ ID NOs:299 and 300. Additional studies using rapid amplification of cDNA ends (RACE), a 5' specific primer to one of the splice forms of B11Ag1 provided above and a breast adenocarcinoma, led to the isolation of three additional, related, splice forms referred to as isoforms B11C-15, B11C-8 and B11C-9,16. The determined cDNA sequences for these isoforms are provided in SEQ ID NO: 301-303, with the corresponding predicted amino acid sequences being provided in SEQ ID NOs:304-306.

In subsequent studies on B305D isoform A (cDNA sequence provided in SEQ ID NO:292), the cDNA sequence (provided in SEQ ID NO:313) was found to contain

an additional guanine residue at position 884, leading to a frameshift in the open reading frame. The determined DNA sequence of this ORF is provided in SEQ ID NO:314. This frameshift generates a protein sequence (provided in SEQ ID NO:315) of 293 amino acids that contains the C-terminal domain common to the other isoforms of B305D but that differs in the N-terminal region.

EXAMPLE 2

PREPARATION OF B18AG1 DNA FROM HUMAN GENOMIC DNA

This Example illustrates the preparation of B18Ag1 DNA by amplification from human genomic DNA.

B18Ag1 DNA may be prepared from 250 ng human genomic DNA using 20 pmol of B18Ag1 specific primers, 500 pmol dNTPS and 1 unit of *Taq* DNA polymerase (Perkin Elmer, Branchburg, NJ) using the following amplification parameters: 94°C for 30 seconds denaturing, 30 seconds 60°C to 42°C touchdown annealing in 2°C increments every two cycles and 72°C extension for 30 seconds. The last increment (a 42°C annealing temperature) should cycle 25 times. Primers were selected using computer analysis. Primers synthesized were B18Ag1-1, B18Ag1-2, B18Ag1-3, and B18Ag1-4. Primer pairs that may be used are 1+3, 1+4, 2+3, and 2+4.

Following gel electrophoresis, the band corresponding to B18Ag1 DNA may be excised and cloned into a suitable vector.

EXAMPLE 3

PREPARATION OF B18AG1 DNA FROM BREAST TUMOR CDNA

This Example illustrates the preparation of B18Ag1 DNA by amplification from human breast tumor cDNA.

First strand cDNA is synthesized from RNA prepared from human breast tumor tissue in a reaction mixture containing 500 ng poly A+ RNA, 200 pmol of the primer

$(T)_{12}AG$ (*i.e.*, TTT TTT TTT TTT AG) (SEQ ID NO:130), 1X first strand reverse transcriptase buffer, 6.7 mM DTT, 500 mmol dNTPs, and 1 unit AMV or MMLV reverse transcriptase (from any supplier, such as Gibco-BRL (Grand Island, NY)) in a final volume of 30 µl. After first strand synthesis, the cDNA is diluted approximately 25 fold and 1 µl is used for amplification as described in Example 2. While some primer pairs can result in a heterogeneous population of transcripts, the primers B18Ag1-2 (5'ATG GCT ATT TTC GGG GGC TGA CA) (SEQ ID NO:126) and B18Ag1-3 (5'CCG GTA TCT CCT CGT GGG TAT T) (SEQ ID NO:127) yield a single 151 bp amplification product.

EXAMPLE 4

IDENTIFICATION OF B-CELL AND T-CELL EPITOPES OF B18AG1

This Example illustrates the identification of B18Ag1 epitopes.

The B18Ag1 sequence can be screened using a variety of computer algorithms. To determine B-cell epitopes, the sequence can be screened for hydrophobicity and hydrophilicity values using the method of Hopp, *Prog. Clin. Biol. Res. 172B*:367-77 (1985) or, alternatively, Cease et al., *J. Exp. Med. 164*:1779-84 (1986) or Spouge et al., *J. Immunol. 138*:204-12 (1987). Additional Class II MHC (antibody or B-cell) epitopes can be predicted using programs such as AMPHI (*e.g.*, Margalit et al., *J. Immunol. 138*:2213 (1987)) or the methods of Rothbard and Taylor (*e.g.*, *EMBO J. 7*:93 (1988)).

Once peptides (15-20 amino acids long) are identified using these techniques, individual peptides can be synthesized using automated peptide synthesis equipment (available from manufacturers such as Perkin Elmer/Applied Biosystems Division, Foster City, CA) and techniques such as Merrifield synthesis. Following synthesis, the peptides can used to screen sera harvested from either normal or breast cancer patients to determine whether patients with breast cancer possess antibodies reactive with the peptides. Presence of such antibodies in breast cancer patient would confirm the immunogenicity of the specific B-cell epitope in question. The peptides can also be tested for their ability to generate a serologic or humoral immune in animals (mice, rats, rabbits,

chimps etc.) following immunization *in vivo*. Generation of a peptide-specific antiserum following such immunization further confirms the immunogenicity of the specific B-cell epitope in question.

To identify T-cell epitopes, the B18Ag1 sequence can be screened using different computer algorithms which are useful in identifying 8-10 amino acid motifs within the B18Ag1 sequence which are capable of binding to HLA Class I MHC molecules. (*see, e.g.*, Rammensee et al., *Immunogenetics 41*:178-228 (1995)). Following synthesis such peptides can be tested for their ability to bind to class I MHC using standard binding assays (*e.g.*, Sette et al., *J. Immunol. 153*:5586-92 (1994)) and more importantly can be tested for their ability to generate antigen reactive cytotoxic T-cells following *in vitro* stimulation of patient or normal peripheral mononuclear cells using, for example, the methods of Bakker et al., *Cancer Res. 55*:5330-34 (1995); Visseren et al., *J. Immunol. 154*:3991-98 (1995); Kawakami et al., *J. Immunol. 154*:3961-68 (1995); and Kast et al., *J. Immunol. 152*:3904-12 (1994). Successful *in vitro* generation of T-cells capable of killing autologous (bearing the same Class I MHC molecules) tumor cells following *in vitro* peptide stimulation further confirms the immunogenicity of the B18Ag1 antigen. Furthermore, such peptides may be used to generate murine peptide and B18Ag1 reactive cytotoxic T-cells following *in vivo* immunization in mice rendered transgenic for expression of a particular human MHC Class I haplotype (Vitiello et al., *J. Exp. Med. 173*:1007-15 (1991).

A representative list of predicted B18Ag1 B-cell and T-cell epitopes, broken down according to predicted HLA Class I MHC binding antigen, is shown below:

Predicted Th Motifs (B-cell epitopes) (SEQ ID NOS.: 131-133)

SSGGRTFDDFHRYLLVGI

QGAAQKPINLSKXIEVVQGHDE

SPGVFLEHLQEAYRIYTPFDLSA

Predicted HLA A2.1 Motifs (T-cell epitopes) (SEQ ID NOS.: 134-140)

YLLVGIQGA

GAAQKPINL

NLSKXIEVV

EVVQGHDES

HLQEAYRIY

NLAFVAQAA

FVAQAAPDS

EXAMPLE 5

IDENTIFICATION OF T-CELL EPITOPES OF B11AG1

This Example illustrates the identification of B11Ag1 (also referred to as B305D) epitopes. Four peptides, referred to as B11-8, B11-1, B11-5 and B11-12 (SEQ ID NOs:309-312, respectfully) were derived from the B11Ag1 gene.

Human CD8 T cells were primed *in vitro* to the peptide B11-8 using dendritic cells according to the protocol of Van Tsai et al. (*Critical Reviews in Immunology 18*:65-75, 1998). The resulting CD8 T cell cultures were tested for their ability to recognize the B11-8 peptide or a negative control peptide, presented by the B-LCL line, JY. Briefly, T cells were incubated with autologous monocytes in the presence of 10 ug/ml peptide, 10 ng/ml IL-7 and 10 ug/ml IL-2, and assayed for their ability to specifically lyse target cells in a standard 51-Cr release assay. As shown in Fig. 22, the bulk culture line demonstrated strong recognition of the B11-8 peptide with weaker recognition of the peptide B11-1.

A clone from this CTL line was isolated following rapid expansion using the monoclonal antibody OKT3 and human IL-2. As shown in Fig. 23, this clone (referred to as A1), in addition to being able to recognize specific peptide, recognized JY LCL transduced with the B11Ag1 gene. This data demonstrates that B11-8 is a naturally processed epitope of the B11Ag1 gene. In addition these T cells were further found to recognize and lyse, in an HLA-A2 restricted manner, an established tumor cell line

naturally expressing B11Ag1 (Fig. 24). The T cells strongly recognize a lung adenocarcinoma (LT-140-22) naturally expressing B11Ag1 transduced with HLA-A2, as well as an A2+ breast carcinoma (CAMA-1) transduced with B11Ag1, but not untransduced lines or another negative tumor line (SW620).

These data clearly demonstrate that these human T cells recognize not only B11-specific peptides but also transduced cells, as well as naturally expressing tumor lines.

CTL lines raised against the antigens B11-5 and B11-12, using the procedures described above, were found to recognize corresponding peptide-coated targets.

EXAMPLE 6

CHARACTERIZATION OF BREAST TUMOR GENES DISCOVERED BY DIFFERENTIAL DISPLAY PCR

The specificity and sensitivity of the breast tumor genes discovered by differential display PCR were determined using RT-PCR. This procedure enabled the rapid evaluation of breast tumor gene mRNA expression semiquantitatively without using large amounts of RNA. Using gene specific primers, mRNA expression levels in a variety of tissues were examined, including 8 breast tumors, 5 normal breasts, 2 prostate tumors, 2 colon tumors, 1 lung tumor, and 14 other normal adult human tissues, including normal prostate, colon, kidney, liver, lung, ovary, pancreas, skeletal muscle, skin, stomach and testes.

To ensure the semiquantitative nature of the RT-PCR, β-actin was used as internal control for each of the tissues examined. Serial dilutions of the first strand cDNAs were prepared and RT-PCR assays performed using β-actin specific primers. A dilution was then selected that enabled the linear range amplification of β-actin template, and which was sensitive enough to reflect the difference in the initial copy number. Using this condition, the β-actin levels were determined for each reverse transcription reaction from each tissue. DNA contamination was minimized by DNase treatment and by assuring a negative result when using first strand cDNA that was prepared without adding reverse transcriptase.

Using gene specific primers, the mRNA expression levels were determined in a variety of tissues. To date, 38 genes have been successfully examined by RT-PCR, five of which exhibit good specificity and sensitivity for breast tumors (B15AG-1, B31GA1b, B38GA2a, B11A1a and B18AG1a). Figures 21A and 21B depict the results for three of these genes: B15AG-1 (SEQ ID NO:27), B31GA1b (SEQ ID NO:148) and B38GA2a (SEQ ID NO:157). Table I summarizes the expression level of all the genes tested in normal breast tissue and breast tumors, and also in other tissues.

TABLE I

Percentage of Breast Cancer Antigens that are Expressed in Various Tissues

Breast Tissues	Over-expressed in Breast Tumors	84%
	Equally Expressed in Normals and Tumor	16%
Other Tissues	Over-expressed in Breast Tumors but not in any Normal Tissues	9%
	Over-expressed in Breast Tumors but Expressed in Some Normal Tissues	30%
	Over-expressed in Breast Tumors but Equally Expressed in All Other Tissues	61%

EXAMPLE 7

PREPARATION AND CHARACTERIZATION OF ANTIBODIES AGAINST BREAST TUMOR POLYPEPTIDES

Polyclonal antibodies against the breast tumor antigen B305D were prepared as follows.

The breast tumor antigen expressed in an *E. coli* recombinant expression system was grown overnight in LB broth with the appropriate antibiotics at 37 °C in a shaking incubator. The next morning, 10 ml of the overnight culture was added to 500 ml to 2x YT plus appropriate antibiotics in a 2L-baffled Erlenmeyer flask. When the Optical Density (at 560 nm) of the culture reached 0.4-0.6, the cells were induced with IPTG (1 mM). Four hours after induction with IPTG, the cells were harvested by centrifugation. The cells were then washed with phosphate buffered saline and centrifuged again. The supernatant was discarded and the cells were either frozen for future use or immediately processed. Twenty ml of lysis buffer was added to the cell pellets and vortexed. To break open the *E. coli* cells, this mixture was then run through the French Press at a pressure of

16,000 psi. The cells were then centrifuged again and the supernatant and pellet were checked by SDS-PAGE for the partitioning of the recombinant protein. For proteins that localized to the cell pellet, the pellet was resuspended in 10 mM Tris pH 8.0, 1% CHAPS and the inclusion body pellet was washed and centrifuged again. This procedure was repeated twice more. The washed inclusion body pellet was solubilized with either 8 M urea or 6 M guanidine HCl containing 10 mM Tris pH 8.0 plus 10 mM imidazole. The solubilized protein was added to 5 ml of nickel-chelate resin (Qiagen) and incubated for 45 min to 1 hour at room temperature with continuous agitation. After incubation, the resin and protein mixture were poured through a disposable column and the flow through was collected. The column was then washed with 10-20 column volumes of the solubilization buffer. The antigen was then eluted from the column using 8M urea, 10 mM Tris pH 8.0 and 300 mM imidazole and collected in 3 ml fractions. A SDS-PAGE gel was run to determine which fractions to pool for further purification.

As a final purification step, a strong anion exchange resin such as HiPrepQ (Biorad) was equilibrated with the appropriate buffer and the pooled fractions from above were loaded onto the column. Antigen was eluted off the column with a increasing salt gradient. Fractions were collected as the column was run and another SDS-PAGE gel was run to determine which fractions from the column to pool. The pooled fractions were dialyzed against 10 mM Tris pH 8.0. The protein was then vialed after filtration through a 0.22 micron filter and the antigens were frozen until needed for immunization.

Four hundred micrograms of B305D antigen was combined with 100 micrograms of muramyldipeptide (MDP). Every four weeks rabbits were boosted with 100 micrograms mixed with an equal volume of Incomplete Freund's Adjuvant (IFA). Seven days following each boost, the animal was bled. Sera was generated by incubating the blood at 4 °C for 12-24 hours followed by centrifugation.

Ninety-six well plates were coated with B305D antigen by incubating with 50 microliters (typically 1 microgram) of recombinant protein at 4 °C for 20 hours. 250 microliters of BSA blocking buffer was added to the wells and incubated at room temperature for 2 hours. Plates were washed 6 times with PBS/0.01% Tween. Rabbit sera

was diluted in PBS. Fifty microliters of diluted sera was added to each well and incubated at room temperature for 30 min. Plates were washed as described above before 50 microliters of goat anti-rabbit horse radish peroxidase (HRP) at a 1:10000 dilution was added and incubated at room temperature for 30 min. Plates were again washed as described above and 100 microliters of TMB microwell peroxidase substrate was added to each well. Following a 15 min incubation in the dark at room temperature, the colorimetric reaction was stopped with 100 microliters of 1N H_2SO_4 and read immediately at 450 nm. The polyclonal antibodies showed immunoreactivity to B305D.

Immunohistochemical (IHC) analysis of B305D expression in breast cancer and normal breast specimens was performed as follows. Paraffin-embedded formal fixed tissue was sliced into 8 micron sections. Steam heat induced epitope retrieval (SHIER) in 0.1 M sodium citrate buffer (pH 6.0) was used for optimal staining conditions. Sections were incubated with 10% serum/PBS for 5 minutes. Primary antibody was added to each section for 25 min at indicated concentrations followed by a 25 min incubation with either an anti-rabbit or anti-mouse biotinylated antibody. Endogenous peroxidase activity was blocked by three 1.5 min incubations with hydrogen peroxide. The avidin biotin complex/horseradish peroxidase (ABC/HRP) systems was used along with DAB chromagen to visualize antigen expression. Slides were counterstained with hematoxylin. B305D expression was detected in both breast tumor and normal breast tissue. However, the intensity of staining was much less in normal samples than in tumor samples and surface expression of B305D was observed only in breast tumor tissues.

A summary of real-time PCR and immunohistochemical analysis of B305D expression in an extensive panel of normal tissues is presented in Table II below. These results demonstrate minimal expression of B305D in testis, inconclusive results in gall bladder, and no detection in all other tissues tested.

TABLE II

mRNA	IHC staining	Tissue type	Summary
Moderately positive	Positive	Testis	Nuclear staining of small minority of spermatids; spermatozoa negative; siminoma negative
Negative	Negative	Thymus	No expression
N/A	Negative	Artery	No expression
Negative	Negative	Skeletal muscle	No expression
Negative	Positive (weak staining)	Small bowel	No expression
Negative	Positive (weak staining)	Ovary	No expression
Negative		Pituitary	No expression
Negative	Positive (weak staining)	Stomach	No expression
Negative	Negative	Spinal cord	No expression
Negative	Negative	Spleen	No expression
Negative	Negative	Ureter	No expression
N/A	Negative	Gall bladder	Inconclusive
N/A	Negative	Placenta	No expression
Negative	Negative	Thyroid	No expression
Negative	Negative	Heart	No expression
Negative	Negative	Kidney	No expression
Negative	Negative	Liver	No expression
Negative	Negative	Brain-cerebellum	No expression
Negative	Negative	Colon	No expression
Negative	Negative	Skin	No expression
Negative	Negative	Bone marrow	No expression
N/A	Negative	Parathyroid	No expression
Negative	Negative	Lung	No expression
Negative	Negative	Esophagus	No expression
Negative	Positive (weak staining)	Uterus	No expression
Negative	Negative	Adrenal	No expression
Negative	Negative	Pancreas	No expression
N/A	Negative	Lymph node	No expression
Negative	Negative	Brain-cortex	No expression
N/A	Negative	Fallopian tube	No expression
Negative	Positive (weak staining)	Bladder	No expression
Negative	N/A	Bone	No expression
Negative	N/A	Salivary gland	No expression
Negative	N/A	Activated PBMC	No expression
Negative	N/A	Resting PBMC	No expression
Negative	N/A	Trachea	No expression
Negative	N/A	Vena cava	No expression

Negative	N/A	Retina	No expression
Negative	N/A	Cartilage	No expression

EXAMPLE 8

PROTEIN EXPRESSION OF BREAST TUMOR ANTIGENS

This example describes the expression and purification of the breast tumor antigen B305D in *E. coli* and in mammalian cells.

Expression of B305D isoform C-15 (SEQ ID NO:301; translated to 384 amino acids) in *E. coli* was achieved by cloning the open reading frame of B305D isoform C-15 downstream of the first 30 amino acids of the *M. tuberculosis* antigen Ra12 (SEQ ID NO:318) in pET17b. First, the internal EcoRI site in the B305D ORF was mutated without changing the protein sequence so that the gene could be cloned at the EcoRI site with Ra12. The PCR primers used for site-directed mutagenesis are shown in SEQ ID NO:319 (referred to as AW012) and SEQ ID NO:320 (referred to as AW013). The ORF of EcoRI site-modified B305D was then amplified by PCR using the primers AW014 (SEQ ID NO:321) and AW015 (SEQ ID NO:322). The PCR product was digested with EcoRI and ligated to the Ra12/pET17b vector at the EcoRI site. The sequence of the resulting fusion construct (referred to as Ra12mB11C) was confirmed by DNA sequencing. The determined cDNA sequence for the fusion construct is provided in SEQ ID NO:323, with the amino acid sequence being provided in SEQ ID NO:324.

The fusion construct was transformed into BL21(DE3)CodonPlus-RIL *E. coli* (Stratagene) and grown overnight in LB broth with kanamycin. The resulting culture was induced with IPTG. Protein was transferred to PVDF membrane and blocked with 5% non-fat milk (in PBS-Tween buffer), washed three times and incubated with mouse anti-His tag antibody (Clontech) for 1 hour. The membrane was washed 3 times and probed with HRP-Protein A (Zymed) for 30 min. Finally, the membrane was washed 3 times and developed with ECL (Amersham). Expression was detected by Western blot.

For recombinant expression in mammalian cells, B305D isoform C-15 (SEQ ID NO:301; translated to 384 amino acids) was subcloned into the mammalian expression vectors pCEP4 and pcDNA3.1 (Invitrogen). These constructs were transfected into HEK293 cells (ATCC) using Fugene 6 reagent (Roche). Briefly, the HEK cells were plated at a density of 100,000 cells/ml in DMEM (Gibco) containing 10% FBS (Hyclone) and grown overnight. The following day, 2 ul of Fugene 6 was added to 100 ul of DMEM containing no FBS and incubated for 15 minutes at room temperature. The Fugene 6/DMEM mixture was added to 1 ug of B305D/pCEP4 or B305D/pcDNA plasmid DNA and incubated for 15 minutes at room temperature. The Fugene/DNA mix was then added to the HEK293 cells and incubated for 48-72 hours at 37 °C with 7% CO_2. Cells were rinsed with PBS, the collected and pelleted by centrifugation.

For Western blot analysis, whole cell lysates were generated by incubating the cells in Triton-X100 containing lysis buffer for 30 minutes on ice. Lysates were then cleared by centrifugation at 10,000 rpm for 5 minutes at 4 °C. Samples were diluted with SDS_PAGE loading buffer containing beta-mercaptoethanol, and boiled for 10 minutes prior to loading the SDS_PAGE gel. Proteins were transferred to nitrocellulose and probed using Protein A purified anti-B305D rabbit polyclonal sera (prepared as described above) at a concentration of 1 ug/ml. The blot was revealed with a goat anti-rabbit Ig coupled to HRP followed by incubation in ECL substrate. Expression of B305D was detected in the the HEK293 lysates transfected with B305D, but not in control HEK293 cells transfected with vector alone.

For FACS analysis, cells were washed further with ice cold staining buffer and then incubated with a 1:100 dilution of a goat anti-rabbit Ig (H+L)-FITC reagent (Southern Biotechnology) for 30 minutes on ice. Following 3 washes, the cells were resuspended in staining buffer containing Propidium Iodide (PI), a vital stain that allows for identification of permeable cells, and then analyzed by FACS. The FACS analysis showed surface expression of B305D protein.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

CLAIMS

What is claimed:

1. An isolated polypeptide, comprising at least an immunogenic portion of a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317;

(b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and

(c) complements of sequences of (a) or (b).

2. An isolated polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences.

3. An isolated polypeptide comprising a sequence recited in any one of SEQ ID NOs: 299, 300, 304-306, 308-312 and 314.

4. An isolated polynucleotide encoding at least 15 amino acid residues of a breast tumor protein, or a variant thereof that differs in one or more substitutions, deletions,

additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317, or a complement of any of the foregoing sequences.

5. An isolated polynucleotide encoding a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing sequences.

6. An isolated polynucleotide, comprising a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317.

7. An isolated polynucleotide, comprising a sequence that hybridizes to a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions.

8. An isolated polynucleotide complementary to a polynucleotide according to any one of claims 4-7.

9. An expression vector, comprising a polynucleotide according to any one of claims 4-8.

10. A host cell transformed or transfected with an expression vector according to claim 9.

11. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a breast tumor protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences.

12. A fusion protein, comprising at least one polypeptide according to claim 1.

13. A fusion protein according to claim 12, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.

14. A fusion protein according to claim 12, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.

15. A fusion protein according to claim 12, wherein the fusion protein comprises an affinity tag.

16. An isolated polynucleotide encoding a fusion protein according to claim 12.

17. A pharmaceutical composition, comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:

(a) a polypeptide according to claim 1;

(b) a polynucleotide according to claim 4;

(c) an antibody according to claim 11;

(d) a fusion protein according to claim 12; and

(e) a polynucleotide according to claim 16.

18. An immunogenic composition comprising an immunostimulant and at least one component selected from the group consisting of:

(a) a polypeptide according to claim 1;

(b) a polynucleotide according to claim 4;

(c) an antibody according to claim 11;

(d) a fusion protein according to claim 12; and

(e) a polynucleotide according to claim 16.

19. An immunogenic composition according to claim 18, wherein the immunostimulant is an adjuvant.

20. An immunogenic composition according to claim 18, wherein the immunostimulant induces a predominantly Type I response.

21. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a pharmaceutical composition according to claim 17.

22. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of an immunogenic composition according to claim 18.

23. A pharmaceutical composition comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with a pharmaceutically acceptable carrier or excipient.

24. A pharmaceutical composition according to claim 23, wherein the antigen presenting cell is a dendritic cell or a macrophage.

25. An immunogenic composition comprising an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317;

(b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and

(c) complements of sequences of (i) or (ii);

in combination with an immunostimulant.

26. An immunogenic composition according to claim 25, wherein the immunostimulant is an adjuvant.

27. An immunogenic composition according to claim 25, wherein the immunostimulant induces a predominantly Type I response.

28. An immunogenic composition according to claim 25, wherein the antigen-presenting cell is a dendritic cell.

29. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317;

(b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and

(c) complements of sequences of (i) or (ii)encoded by a polynucleotide recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317;

and thereby inhibiting the development of a cancer in the patient.

30. A method according to claim 29, wherein the antigen-presenting cell is a dendritic cell.

31. A method according to any one of claims 21, 22 and 29, wherein the cancer is breast cancer.

32. A method for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) polynucleotides recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317; and

(ii) complements of the foregoing polynucleotides;

wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

33. A method according to claim 32, wherein the biological sample is blood or a fraction thereof.

34. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated according to the method of claim 32.

35. A method for stimulating and/or expanding T cells specific for a breast tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

(a) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) sequences recited in SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317;

(ii) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and

(iii) complements of sequences of (i) or (ii);

(b) polynucleotides encoding a polypeptide of (a); and

(c) antigen presenting cells that express a polypeptide of (a);

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

36. An isolated T cell population, comprising T cells prepared according to the method of claim 35.

37. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population according to claim 36.

38. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating $CD4^+$ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(1) sequences recited in SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317;

(2) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and

(3) complements of sequences of (1) or (2);

(ii) polynucleotides encoding a polypeptide of (i); and

(iii) antigen presenting cells that expresses a polypeptide of (i);

such that T cells proliferate; and

(b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.

39. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating $CD4^+$ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(1) sequences recited in SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317;

(2) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and

(3) complements of sequences of (1) or (2);

(ii) polynucleotides encoding a polypeptide of (i); and

(iii) antigen presenting cells that express a polypeptide of (i);

such that T cells proliferate;

(b) cloning at least one proliferated cell to provide cloned T cells; and

(c) administering to the patient an effective amount of the cloned T cells, and thereby inhibiting the development of a cancer in the patient.

40. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with a binding agent that binds to a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(c) comparing the amount of polypeptide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

41. A method according to claim 40, wherein the binding agent is an antibody.

42. A method according to claim 43, wherein the antibody is a monoclonal antibody.

43. A method according to claim 40, wherein the cancer is breast cancer.

44. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polypeptide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

45. A method according to claim 44, wherein the binding agent is an antibody.

46. A method according to claim 45, wherein the antibody is a monoclonal antibody.

47. A method according to claim 44, wherein the cancer is a breast cancer.

48. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and

(c) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

49. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

50. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

51. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NO: 1, 3-86, 142-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polynucleotide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

52. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

53. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

54. A diagnostic kit, comprising:

(a) one or more antibodies according to claim 11; and

(b) a detection reagent comprising a reporter group.

55. A kit according to claim 54, wherein the antibodies are immobilized on a solid support.

56. A kit according to claim 54, wherein the detection reagent comprises an anti-immunoglobulin, protein G, protein A or lectin.

57. A kit according to claim 54, wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

58. An oligonucleotide comprising 10 to 40 contiguous nucleotides that hybridize under moderately stringent conditions to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288,

291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotides.

59. A oligonucleotide according to claim 58, wherein the oligonucleotide comprises 10-40 contiguous nucleotides recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317.

60. A diagnostic kit, comprising:

(a) an oligonucleotide according to claim 59; and

(b) a diagnostic reagent for use in a polymerase chain reaction or hybridization assay.

COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER

ABSTRACT OF THE DISCLOSURE

Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA encoding such a protein, in a sample are also provided.

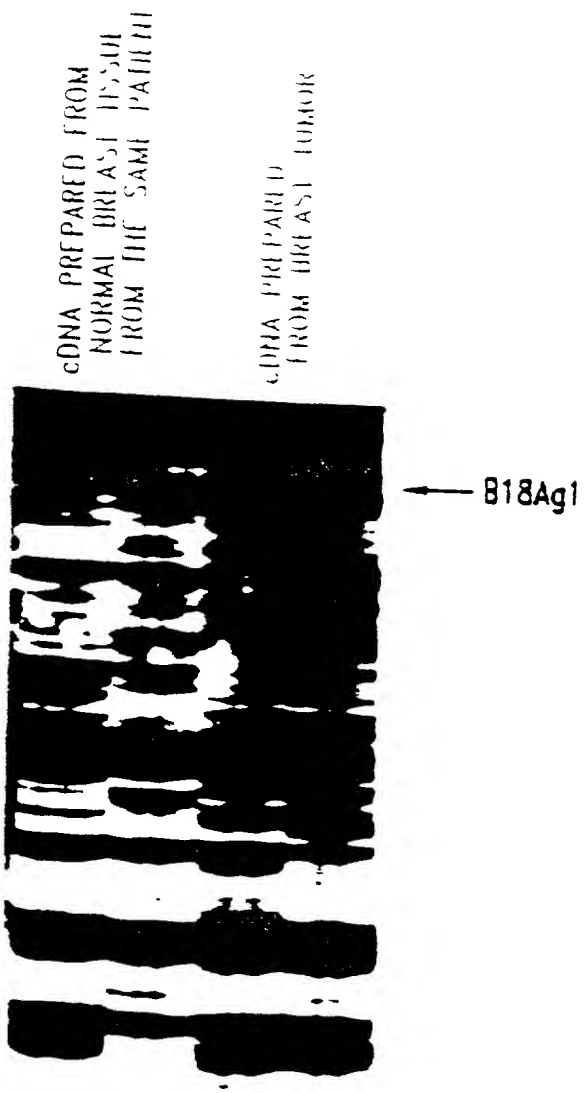
cDNA PREPARED FROM
NORMAL BREAST TISSUE
FROM THE SAME PATIENT
cDNA PREPARED
FROM BREAST TUMOR
B18Ag1

Fig. 1

Fig. 2

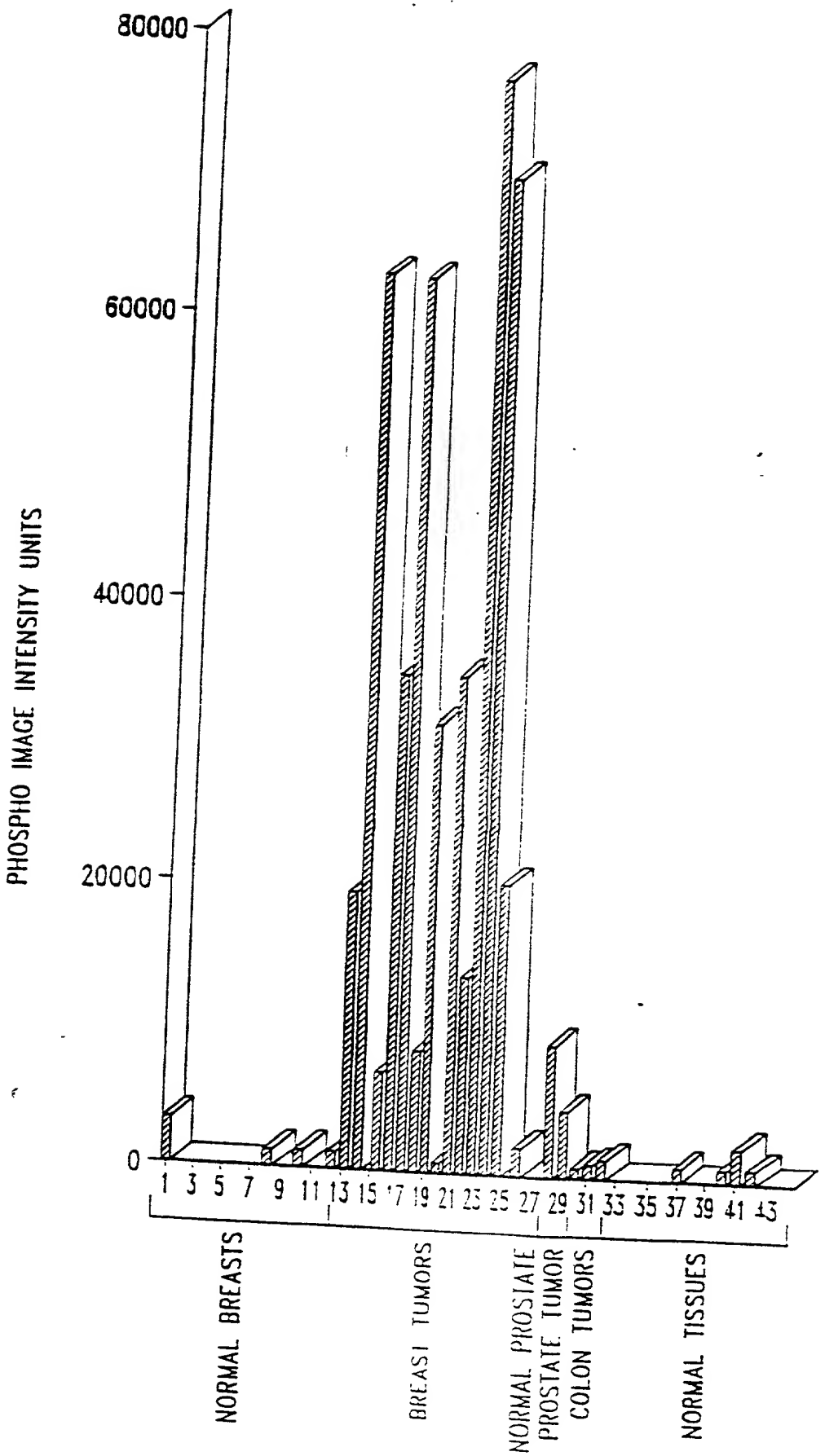
80000
60000
40000
20000
0
PHOSPHO IMAGE INTENSITY UNITS
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
NORMAL BREASTS
BREAST TUMORS
NORMAL PROSTATE
PROSTATE TUMOR
COLON TUMORS
NORMAL TISSUES

Fig. 3

GENOMIC CLONE MAP

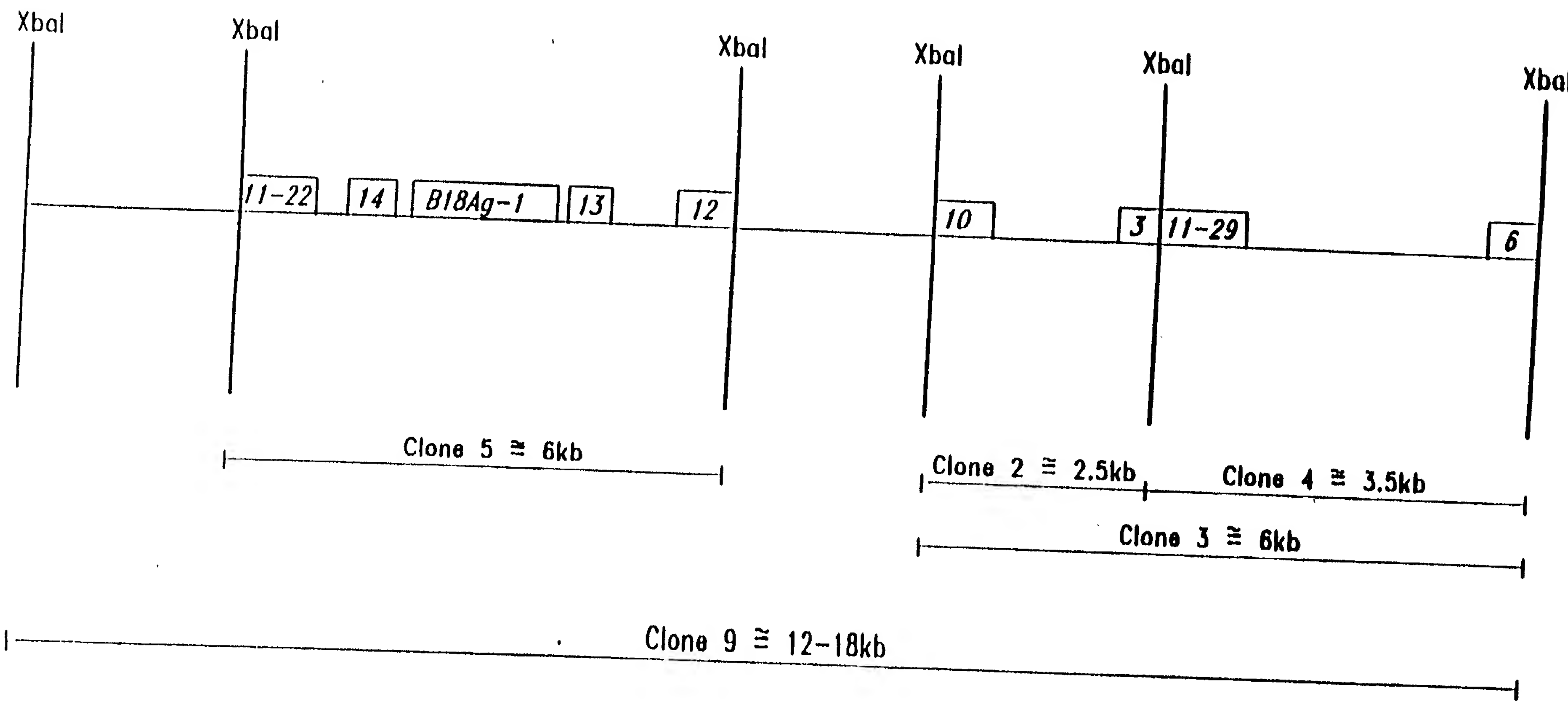

Fig. 4

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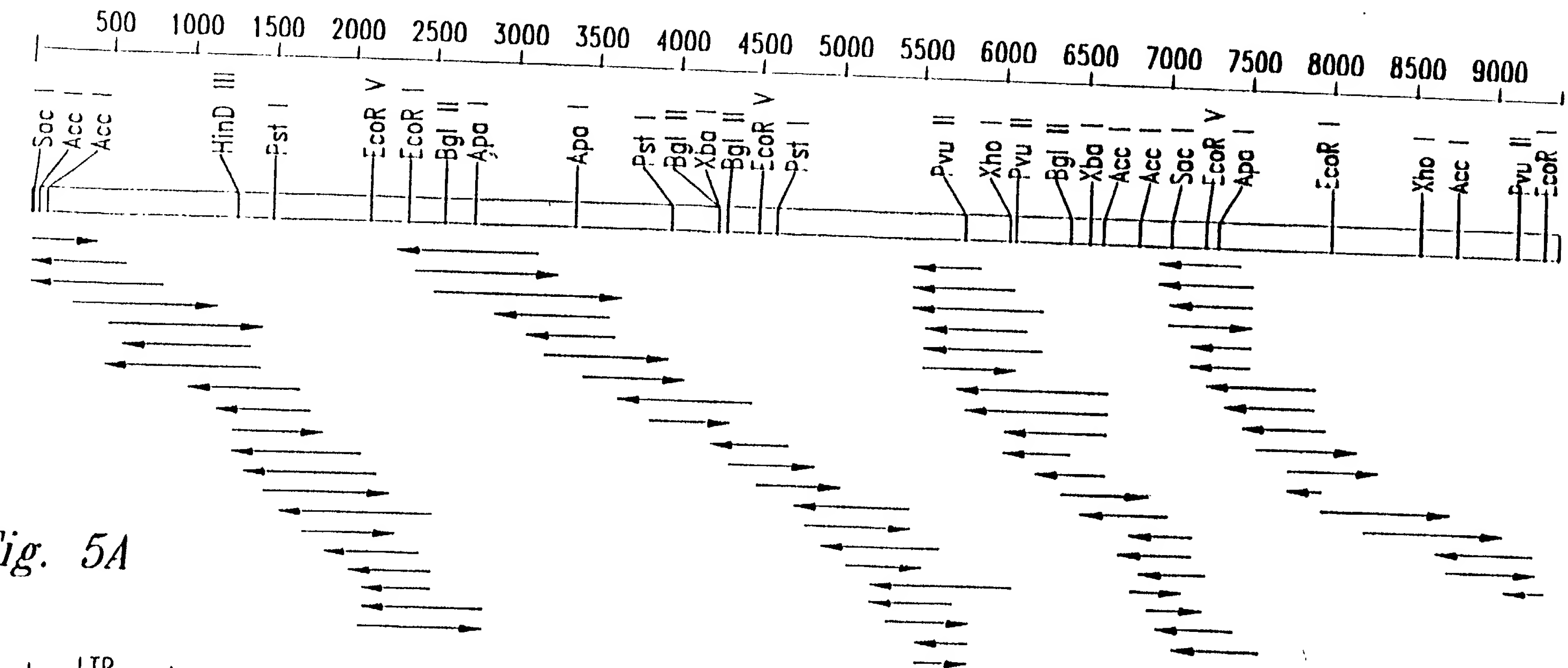

Fig. 5A

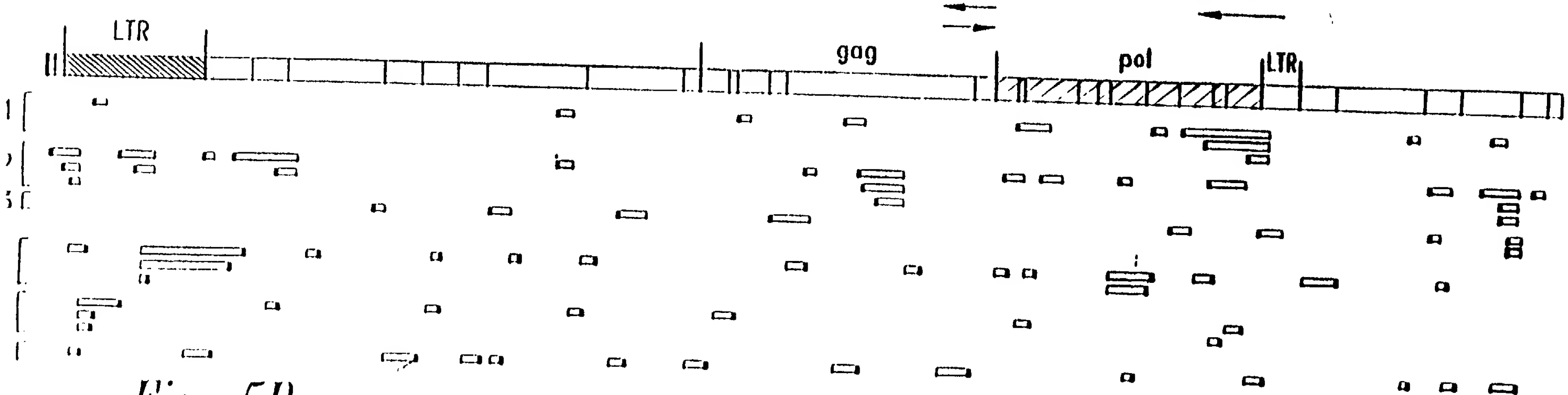

Fig. 5B

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B18Ag1

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Leu Glu Thr Gln Leu Gly Pro Asn Trp Asp Pro Asn Phe Ser Ser Gly
  1               5                  10                  15

GGG AGA ACT TTT GAC GAT TTC CAC CGG TAT CTC CTC GTG GGT ATT CAG   96
Gly Arg Thr Phe Asp Asp Phe His Arg Tyr Leu Leu Val Gly Ile Gln
            20                  25                  30

GGA GCT GCC CAG AAA CCT ATA AAC TTG TCT AAG GCG ATT GAA GTC GTC  144
Gly Ala Ala Gln Lys Pro Ile Asn Leu Ser Lys Ala Ile Glu Val Val
        35                  40                  45

CAG GGG CAT GAT GAG TCA CCA GGA GTG TTT TTA GAG CAC CTC CAG GAG  192
Gln Gly His Asp Glu Ser Pro Gly Val Phe Leu Glu His Leu Gln Glu
    50                  55                  60

GCT TAT CGG ATT TAC ACC CCT TTT GAC CTG GCA GCC CCC GAA AAT AGC  240
Ala Tyr Arg Ile Tyr Thr Pro Phe Asp Leu Ala Ala Pro Glu Asn Ser
65                  70                  75                  80

CAT GCT CTT AAT TTG GCA TTT GTG GCT CAG GCA GCC CCA GAT AGT AAA  288
His Ala Leu Asn Leu Ala Phe Val Ala Gln Ala Ala Pro Asp Ser Lys
                85                  90                  95

AGG AAA CTC CAA AAA CTA GAG GGA TTT TGC TGG AAT GAA TAC CAG TCA  336
Arg Lys Leu Gln Lys Leu Glu Gly Phe Cys Trp Asn Glu Tyr Gln Ser
            100                 105                 110

GCT TTT AGA GAT AGC CTA AAA GGT TTT                             363
Ala Phe Arg Asp Ser Leu Lys Gly Phe
        115                 120
```

Fig. 6

NUCLEOTIDE SEQUCNE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B17Ag1

GC TGGGCACAGT GGCTCATACC TGTAATCCTG ACCGTTTCAG AGGCTCAGGT	60
CG CTTGAGCCCA AGATTTCAAG ACTAGTCTGG GTAACATAGT GAGACCCTAT	120
AA AAATAAAAAA ATGAGCCTGG TGTAGTGGCA CACACCAGCT GAGGAGGGAG	180
CT AGGAGA	196

Fig. 7

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B17Ag2

GC TTGGGGGCTC TGACTAGAAA TTCAAGGAAC CTGGGATTCA AGTCCAACTG	60
AC TTACACTGTG GNCTCCAATA AACTGCTTCT TTCCTATTCC CTCTCTATTA	120
AA GGAAAACGAT GTCTGTGTAT AGCCAAGTCA GNTATCCTAA AAGGAGATAC	180
AT TAAATATCAG AATGTAAAAC CTGGGAACCA GGTTCCCAGC CTGGGATTAA	240
CA AGAAGACTGA ACAGTACTAC TGTGAAAAGC CCGAAGNGGC AATATGTTCA	300
TT GAAGGATGGC TGGGAGAATG AATGCTCTGT CCCCCAGTCC CAAGCTCACT	360
CT CCTTTATAGC CTAGGAGA	388

Fig. 8

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B13Ag2a

```
GC CTATAATCAT GTTTCTCATT ATTTTCACAT TTTATTAACC AATTTCTGTT    60

AA AATATGAGGG AAATATATGA AACAGGGAGG CAATGTTCAG ATAATTGATC   120

TG ATTTCTACAT CAGATGCTCT TTCCTTTCCT GTTTATTTCC TTTTTATTTC   180

GG TCGAATGTAA TAGCTTTGTT TCAAGAGAGA GTTTTGGCAG TTTCTGTAGC   240

CT GCTCATGTCT CCAGGCATCT ATTTGCACTT TAGGAGGTGT CGTGGGAGAC   300

CT ATTTTTTCCA TATTTGGGCA ACTACTA                            337
```

Fig. 9

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B13Ag1b

GC CATACAGTGC CTTTCCATTT ATTTAACCCC CACCTGAACG GCATAAACTG 60

GC TGGTGTTTTT TACTGTAAAC AATAAGGAGA CTTTGCTCTT CATTTAAACC 120

AT TTCATATTTT ACGCTCGAGG GTTTTTACCG GTTCCTTTTT ACACTCCTTA 180

TT TAAGTCGTTT GGAACAAGAT ATTTTTTCTT TCCTGGCAGC TTTTAACATT 240

TT TGTGTCTGGG GGACTGCTGG TCACTGTTTC TCACAGTTGC AAATCAAGGC 300

CC AAGAAAAAAA AATTTTTTTG TTTTATTTGA AACTGGACCG GATAAACGGT 360

CG GCTGCTGTAT ATAGTTTTAA ATGGTTTATT GCACCTCCTT AAGTTGCACT 420

GG GGGGNTTTTG NATAGAAAGT NTTTANTCAC ANAGTCACAG GGACTTTTNT 480

NA CTGAGCTAAA AAGGGCTGNT TTTCGGGTGG GGGCAGATGA AGGCTCACAG 540

TC TCTTAGAGGG GGGAACTNCT A 571

Fig. 10

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B13Ag1a

```
TA ATAACTTAAA TATATTTTGA TCACCCACTG GGGTGATAAG ACAATAGATA      60
TT TCCAAAAAGC ATAAAACCAA AGTATCATAC CAAACCAAAT TCATACTGCT     120
CC GCACTGAAAC TTCACCTTCT AACTGTCTAC CTAACCAAAT TCTACCCTTC     180
GG TGCGTGCTCA CTACTCTTTT TTTTTTTTTT TTTNTTTTGG AGATGGAGTC     240
CA GCCCAGGGGT GGAGTACAAT GGCACAACCT CAGCTCACTG NAACCTCCGC     300
TT CATGAGATTC TCCTGNTTCA GCCTTCCCAG TAGCTGGGAC TACAGGTGTG     360
TG CCTGGNTAAT CTTTTTTNGT TTTNGGGTAG AGATGGGGGT TTTACATGTT     420
TG GTNTCGAACT CCTGACCTCA AGTGATCCAC CCACCTCAGG CTCCCAAAGT     480
TA CAGACATGAG CCACTGNGCC CAGNCCTGGT GCATGCTCAC TTCTCTAGGC     540

                                                              548
```

Fig. 11

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B11Ag1

```
TG CACATGCAGA ATATTCTATC GGTACTTCAG CTATTACTCA TTTTGATGGC      60

AG CCTATCCTCA AGATGAGTAT TTAGAAAGAA TTGATTTAGC GATAGACCAA     120

GC ACTCTGACTA CACGAAATTG TTCAGATGTG ATGGATTTAT GACAGTTGAT     180

GA GATTATTAAG TGATTATTTT AAAGGGAATC CATTAATTCC AGAATATCTT     240

TC AAGATGATAT AGAAATAGAA CAGAAAGAGA CTACAAATGA AGATGTATCA     300

TA TTGAAGAGCC TATAGTAGAA AATGAATTAG CTGCATTTAT TAGCCTTACA     360

TT TTCCTGATGA ATCTTATATT CAGCCATCGA CATAGCATTA CCTGATGGGC     420

GA ATAATAGAAA CTGGGTGCGG GGCTATTGAT GAATTCATCC NCAGTAAATT     480

AC AAAATATAAC TCGATTGCAT TTGGATGATG GAATACTAAA TCTGGCAAAA     540

GG AGCTACTAGT AACCTCTCTT TTTGAGATGC AAAATTTTCT TTTAGGGTTT     600

CT ACTTTACGGA TATTGGAGCA TAACGGGA                            638
```

Fig. 12

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B3CA3c

```
ACTGATGGAT GTCGCCGGAG GCGAGGGGCC TTATCTGATG CTCGGCTGCC TGTTCGTGAT   60

GTGCGCGGCG ATTGGGCTGT TTATCTCAAA CACCGCCACG GCGGTGCTGA TGGCGCCTAT  120

TGCCTTAGCG GCGGCGAAGT CAATGGGCGT CTCACCCTAT CCTTTTGCCA TGGTGGTGGC  180

GATGGCGGCT TCGGCGGCGT TTATGACCCC GGTCTCCTCG CCGGTTAACA CCCTGGTGCT  240

TGGCCCTGGC AAGTACTCAT TTAGCGATTT TGTCAAAATA GGCGTG                  286
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Fig. 13

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA 89CG1

```
AG CAGCCCCTTC TTCTCAATTT CATCTGTCAC TACCCTGGTG TAGTATCTCA      60

CA TTTTTATAGC CTCCTCCCTG GTCTGTCTTT TGATTTTCCT GCCTGTAATC     120

AC ATAACTGCAA GTAAACATTT CTAAAGTGTG GTTATGCTCA TGTCACTCCT     180

AA ATAGTTTCCA TTACCGTCTT AATAAAATTC GGATTTGTTC TTTNCTATTN     240

CA CCTATGACCG AA                                             262
```

Fig. 14

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B9CG3

```
AG CAAAGCCAGT GGTTTGAGCT CTCTACTGTG TAAACTCCTA AACCAAGGCC      60

TA AATGGTGGCA GGATTTTTAT TATAAACATG TACCCATGCA AATTTCCTAT      120

GA TATATTCTTC TACATTTAAA CAATAAAAAT AATCTATTTT TAAAAGCCTA      180

AG TTAGGTAAGA GTGTTTAATG AGAGGGTATA AGGTATAAAT CACCAGTCAA      240

TG CCTATGACCG A                                               261
```

Fig. 15

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA 82CA2

```
GG GCATGGACGC AGACGCCTGA CGTTTGGCTG AAAATCTTTC ATTGATTCGT      60

AT AGGAAAATTC CCAAAGAGGG AATGTCCTGT TGCTCGCCAG TTTTTNTGTT     120

GG ANAAGGCAAN GAGCTCTTCA GACTATTGGN ATTNTCGTTC GGTCTTCTGC     180

CG NCTTGCNANG ATCTTCAT                                        208
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Fig. 16

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B3CA1

GG GCATGGACGC AGACGCCTGA CGTTTGGCTG AAAATCTTTC ATTGATTCGT 60

AT AGGAAAATTC CCAAAGAGGG AATGTCCTGT TGCTCGCCAG TTTTTNTGTT 120

GG ANAAGGCAAN GAGCTCTTCA GACTATTGGN ATTNTCGTTC GGTCTTCTGC 180

CG NCTTGCNANG ATCTTCAT 208

Fig. 17

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B3CA2

```
GG GCATGGACGC AGACGCCTGA CGTTTGGCTG AAAATCTTTC ATTGATTCGT      60

AT AGGAAAATTC CCAAAGAGGG AATGTCCTGT TGCTCGCCAG TTTTTNTGTT     120

GG ANAAGGCAAN GAGCTCTTCA GACTATTGGN ATTNTCGTTC GGTCTTCTGC     180

CG NCTTGCNANG ATCTTCAT                                        208
```

Fig. 18

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B3CA3

```
AG GGAGCAAGGA GAAGGCATGG AGAGGCTCAN GCTGGTCCTG GCCTACGACT      60

CT GTCGCCGGGG ATGGTGGAGA ACTGAAGCGG GACCTCCTCG AGGTCCTCCG     120

TC NCCGTCCAGG AGGAGGGTCT TTCCGTGGTC TNGGAGGAGC GGGGGGAGAA     180

TC ATGGTCNACA TCCC                                            204
```

Fig. 19

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA 84CA1

```
TC AGGAGCGGGT AGAGTGGCAC CATTGAGGGG ATATTCAAAA ATATTATTTT    60
TG ATAGTTGCTG AGTTTTTCTT TGACCCATGA GTTATATTGG AGTTTATTTT   120
CC AATCGCATGG ACATGTTAGA CTTATTTTCT GTTAATGATT NCTATTTTTA   180
GA TTTGAGAAAT TGGTTNTTAT TATATCAATT TTTGGTATTT GTTGAGTTTG   240
GC TTAGTATGTG ACCA                                          264
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Fig. 20

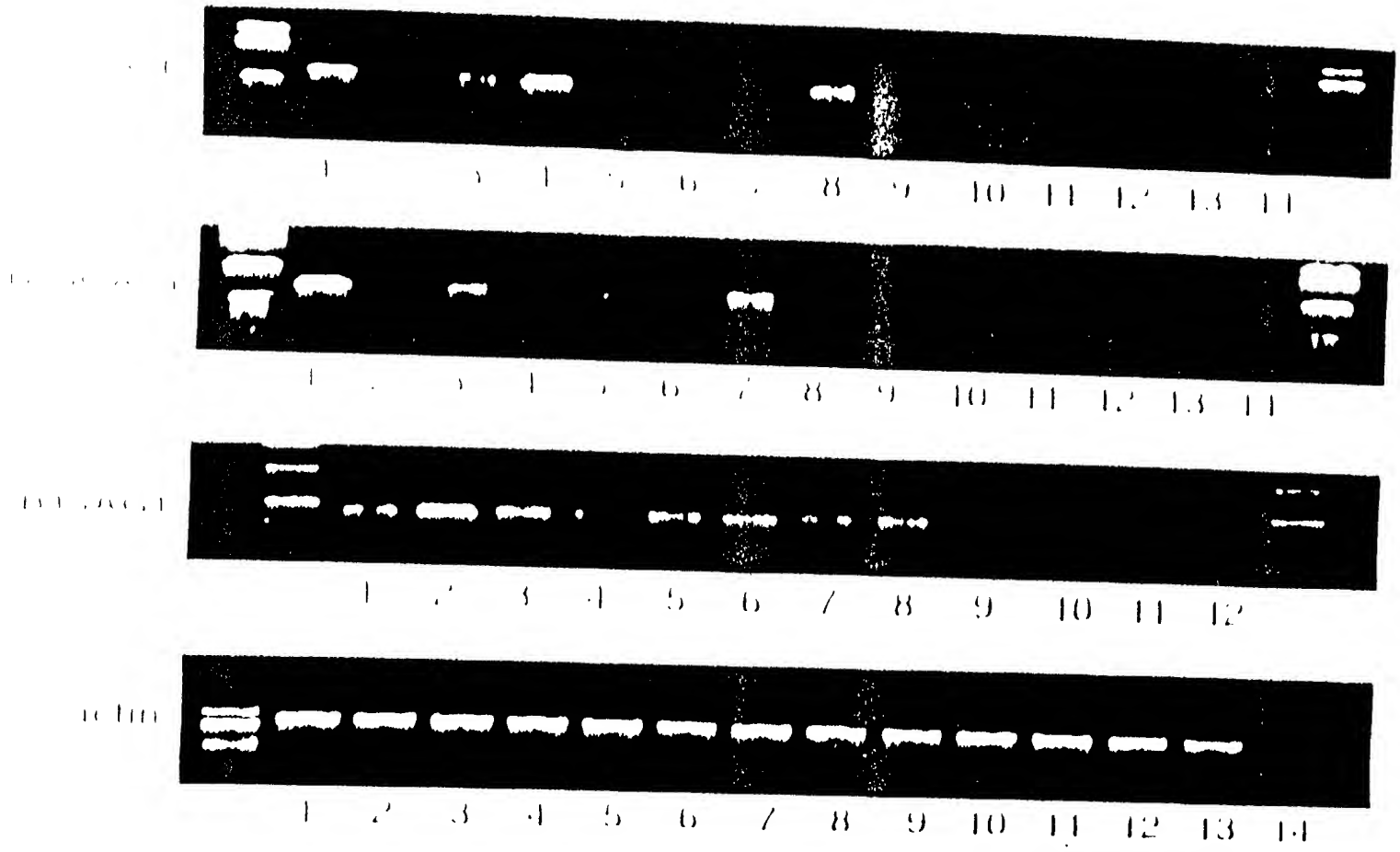
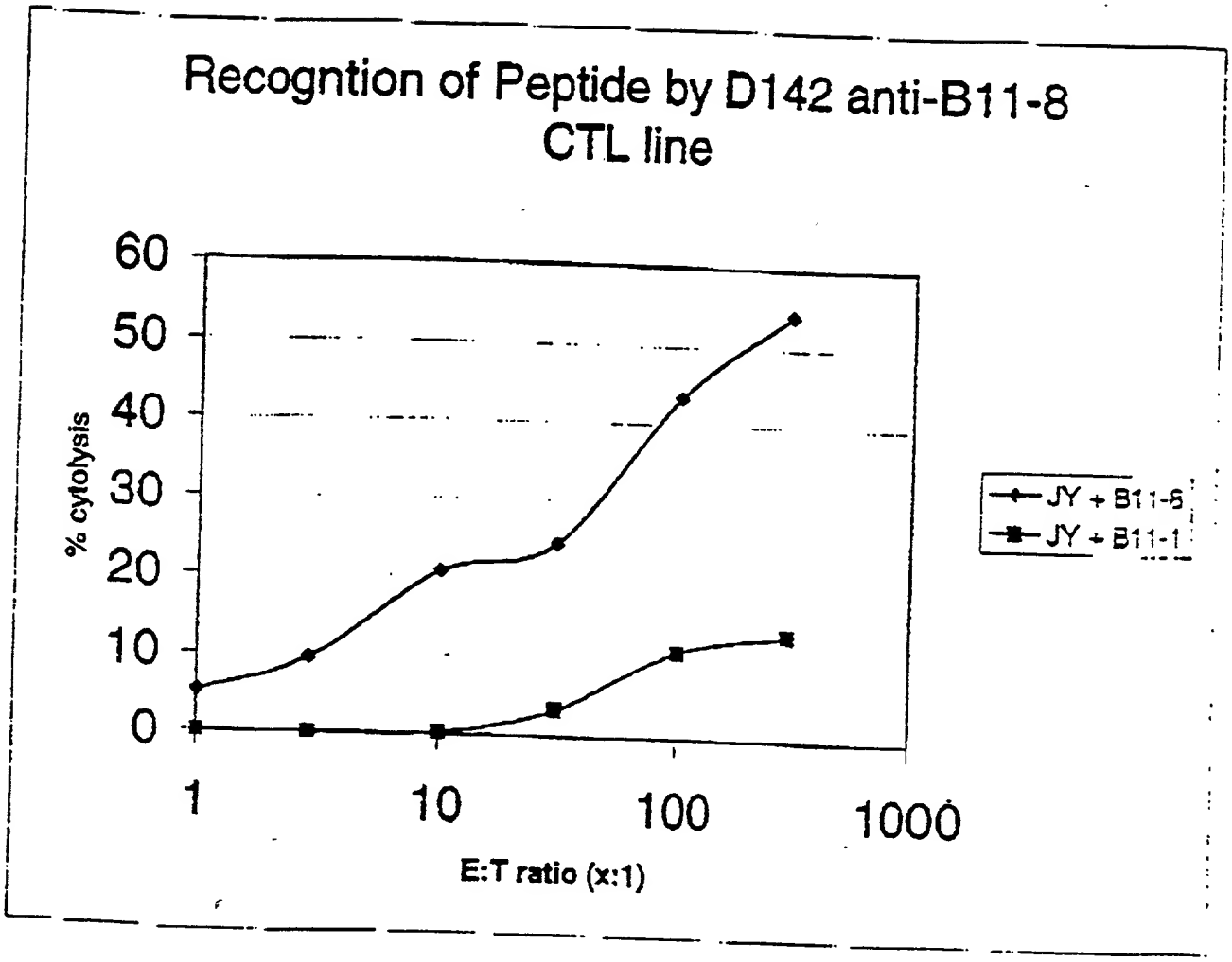


Fig. 21A

Fig. 21B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50
40
30
20
10
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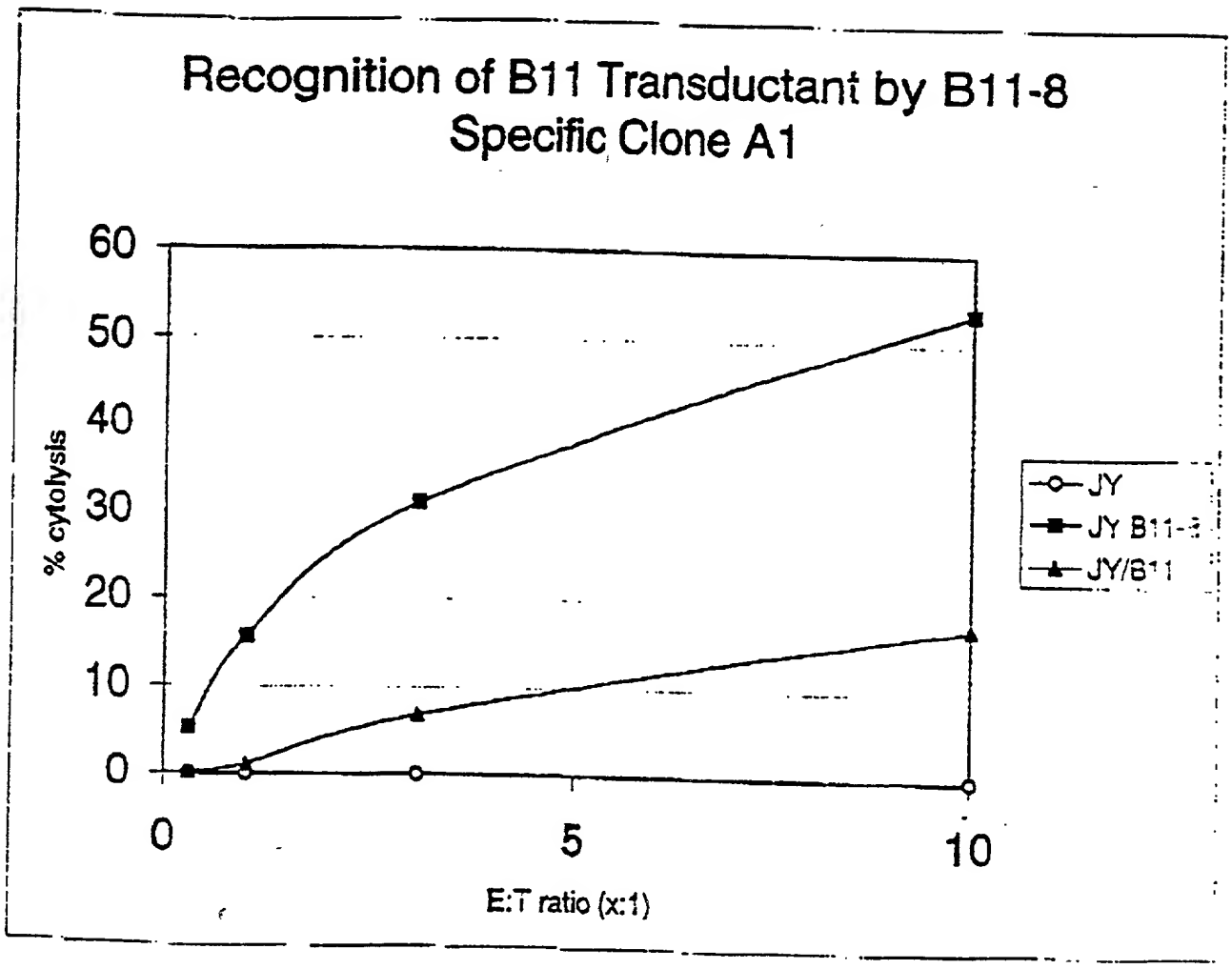
% cytolysis
1
10
100
1000
E:T ratio (x:1)
JY + B11-8
JY + B11-1

Fig. 22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50
40
30
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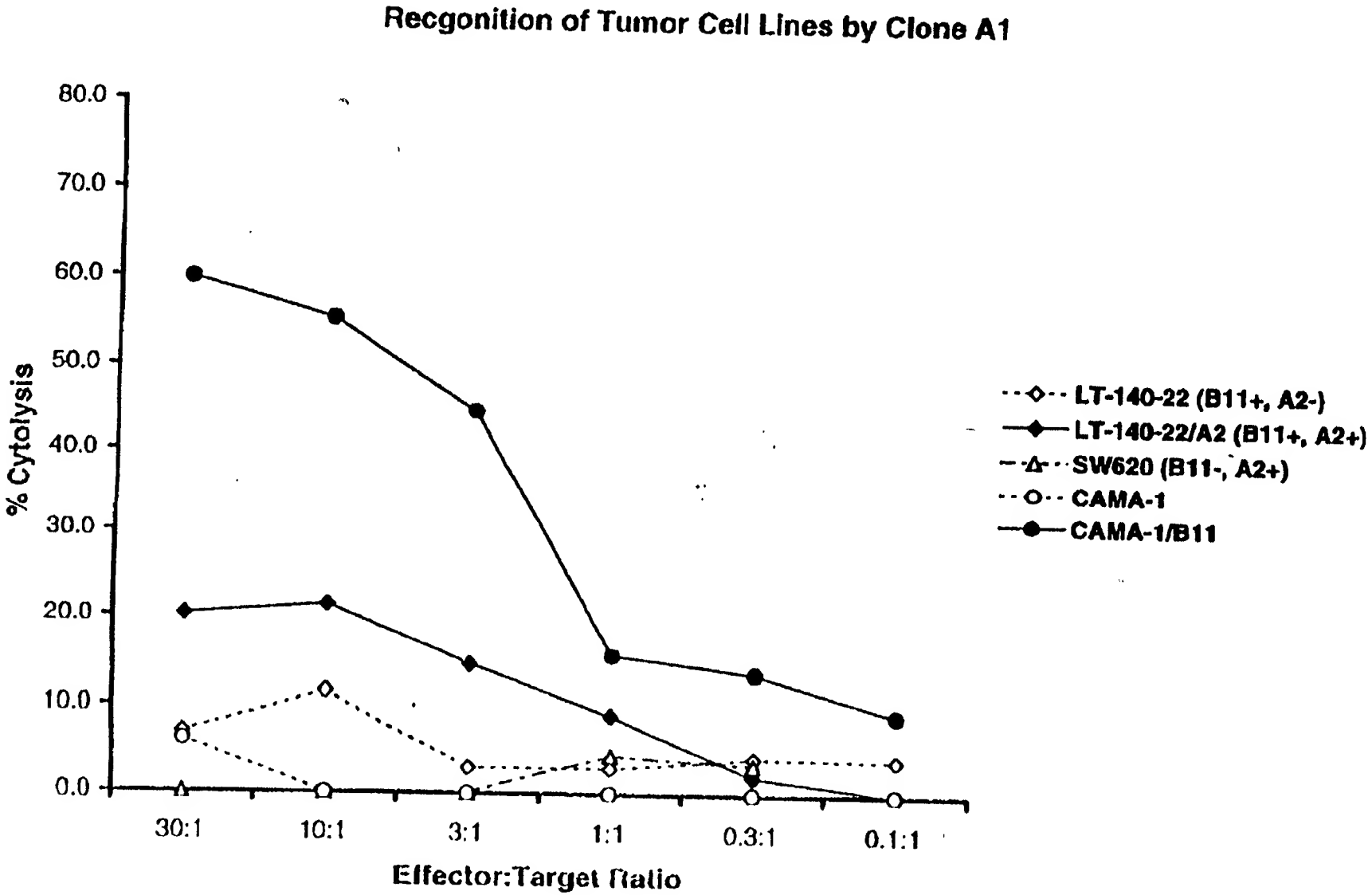
10
0
0
5
10
E:T ratio (x:1)
JY
JY B11-8
JY/B11

Fig. 23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70.0
60.0
50.0
40.0
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20.0
10.0
0.0
% Cytolysis
30:1
10:1
3:1
1:1
0.3:1
0.1:1
Effector:Target Ratio
LT-140-22 (B11+, A2-)
LT-140-22/A2 (B11+, A2+)
SW620 (B11-, A2+)
CAMA-1
CAMA-1/B11

Fig. 24

SEQUENCE LISTING

<110> Frudakis, Tony N.
Reed, Steven G.
Smith, John M.
Misher, Linda E.
Dillon, Davin C.
Retter, Marc W.
Wang, Aijun
Skeiky, Yasir A.W.

<120> COMPOSITIONS AND METHODS FOR THE
THERAPY AND DIAGNOSIS OF BREAST CANCER

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35 40 45

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                85                  90                  95
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<223> n = A,T,C or G

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tctagagctc gcggccgcga gctcaattaa ccctcactaa agggagtcga ctcgatcaga 60
ctgttactgt gtctatgtag aaagaagtag acataagaga ttccattttg ttctgtacta 120
agaaaaattc ttctgccttg agatgctgtt aatctgtaac cctagcccca accctgtgct 180
cacagagaca tgtgctgtgt tgactcaagg ttcaatggat ttagggctat gctttgttaa 240
aaaagtgctt gaagataata tgcttgttaa aagtcatcac cattctctaa tctcaagtac 300
ccagggacac aatacactgc ggaaggccgc agggacctct gtctaggaaa gccaggtatt 360
gtccaagatt tctccccatg tgatagcctg agatatggcc tcatgggaag ggtaagacct 420
gactgtcccc cagcccgaca tcccccagcc cgacatcccc cagcccgaca cccgaaaagg 480
gtctgtgctg aggaagatta ntaaaagagg aaggctcttt gcattgaagt aagaagaagg 540
ctctgtctcc tgctcgtccc tgggcaataa aatgtcttgg tgttaaaccc gaatgtatgt 600
tctacttact gagaatagga gaaaacatcc ttagggctgg aggtgagaca ccctggcggc 660
atactgctct ttaatgcacg agatgtttgt ntaattgcca tccagggcca ncccctttcc 720
ttaacttttt atganacaaa aactttgttc nctttttcctg cgaacctctc cccctattan 780

cctattggcc tgcccatccc ctccccaaan ggtgaaaana tgttcntaaa tncgagggaa 840
tccaaaacnt tttcccgttg gtccccttтc caaccccgtc cctgggccnn tttcctcccc 900
aacntgtccc ggntccttcn ttcccncccc cttcccngan aaaaaacccc gtntganggn 960
gccccctcaa attataacct ttccnaaaca aannggttcn aaggtggttt gnttccggtg 1020
cggctggcct tgaggtcccc cctncacccc aatttggaan ccngtttttt ttattgcccn 1080
ntcccc 1086

<210> 8
<211> 1177
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(1177)
<223> n = A,T,C or G

<400> 8
nccntttaga tgttgacaan ntaaacaagc ngctcaggca gctgaaaaaa gccactgata 60
aagcatcctg gagtatcaga gtttactgtt agatcagcct catttgactt cccctcccac 120
atggtgttta aatccagcta cactacttcc tgactcaaac tccactattc ctgttcatga 180
ctgtcaggaa ctgttggaaa ctactgaaac tggccgacct gatcttcaaa atgtgcccct 240
aggaaaggtg gatgccaccg tgttcacaga cagtaccncc ttcctcgaga agggactacg 300
aggggccggt gcanctgtta ccaaggagac tnatgtgttg tgggctcagg ctttaccanc 360
aaacacctca ncncnnaagg ctgaattgat cgccctcact caggctctcg gatggggtaa 420
gggatattaa cgttaacact gacagcaggt acgcctttgc tactgtgcat gtacgtggag 480
ccatctacca ggagcgtggg ctactcactc ggcaggtggc tgtnatccac tgtaaangga 540
catcaaaagg aaaacnnggc tgttgcccgt ggtaaccana aanctgatcn ncagctcnaa 600
gatgctgtgt tgactttcac tcncncctct taaacttgct gcccacantc tcctttccca 660
accagatctg cctgacaatc cccatactca aaaaaaaaan aanactggcc ccgaacccna 720
accaataaaa acggggangg tnggtnganc nncctgaccc aaaaataatg gatcccccgg 780
gctgcaggaa ttcaattcan ccttatcnat acccccaacn nggngggggg ggccngtncc 840
cattncccct ntattnattc tttnnccccc cccccggcnt ccttttttnaa ctcgtgaaag 900
ggaaaacctg ncttaccaan ttatcncctg gaccntcccc ttccncggtn gnttanaaaa 960
aaaagcccnc antcccntcc naaatttgca cngaaaggna aggaatttaa cctttatttt 1020
ttnntccttt antttgtnnn ccccctttta cccaggcgaa cngccatcnt ttaanaaaaa 1080
aaanagaang tttatttttc cttngaacca tcccaatana aancacccgc nggggaacgg 1140
ggnggnaggc cnctcacccc ctttntgtng gngggnc 1177

<210> 9
<211> 1146
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(1146)
<223> n = A,T,C or G

<400> 9
nccnnttnnt gatgttgtct ttttggcctc tctttggata ctttccctct cttcagaggt 60

gaaaagggtc aaaaggagct gttgacagtc atcccaggtg ggccaatgtg tccagagtac 120
agactccatc agtgaggtca aagcctgggg cttttcagag aagggaggat tatgggtttt 180
ccaattatac aagtcagaag tagaaagaag ggacataaac caggaagggg gtggagcact 240
catcacccag agggacttgt gcctctctca gtggtagtag aggggctact tcctcccacc 300
acggttgcaa ccaagaggca atgggtgatg agcctacagg ggacatancc gaggagacat 360
gggatgaccc taagggagta ggctggtttt aaggcggtgg gactgggtga gggaaactct 420
cctcttcttc agagagaagc agtacagggc gagctgaacc ggctgaaggt cgaggcgaaa 480
acacggtctg gctcaggaag accttggaag taaaattatg aatggtgcat gaatggagcc 540
atggaagggg tgctcctgac caaactcagc cattgatcaa tgttagggaa actgatcagg 600
gaagccggga atttcattaa caacccgcca cacagcttga acattgtgag gttcagtgac 660
ccttcaaggg gccactccac tccaactttg gccattctac tttgcnaaat ttccaaaact 720
tcctttttta aggccgaatc cntantccct naaaaacnaa aaaaaatctg cncctattct 780
ggaaaaggcc canccccttac caggctggaa gaaattttnc cttttttttt tttttgaagg 840
cntttnttaa attgaacctn aattcncccc cccaaaaaaa aacccnccng gggggcggat 900
ttccaaaaac naattccctt accaaaaaac aaaaacccnc ccttnttccc ttccnccctn 960
ttcttttaat tagggagaga tnaagccccc caatttccng gnctngatnn gtttcccccc 1020
cccccatttt ccnaaacttt ttcccancna ggaanccncc ctttttttng gtcngattna 1080
ncaaccttcc aaaccatttt tccnnaaaaa ntttgntngg ngggaaaaan acctnnnttt 1140
atagan 1146

<210> 10
<211> 545
<212> DNA
<213> Homo sapien

<400> 10
cttcattggg tacgggcccc ctcgaggtcg acggtatcga taagcttgat atcgaattcc 60
tgcagcccgg gggatccact agttctagag tcaggaagaa ccaccaacct tcctgatttt 120
tattggctct gagttctgag gccagttttc ttcttctgtt gagtatgcgg gattgtcagg 180
cagatctggc tgtggaaagg agactgtggg cagcaagttt agaggcgtga ctgaaagtca 240
cactgcatct tgagctgctg aatcagcttt ctggttacca cgggcaacag ccgtgttttc 300
cttttgatgt cctttacagt ggattacagc cacctgctga ggtgagtagc ccacgctcct 360
ggtagatggc tccacgtaca tgcacagtag caaaggcgta cctgctgtca gtgttaacgt 420
taatatcctt accccatcgg agagcctgag tgagggcgat caattcagcc cttttgtgct 480
gaggtgtttg ctggttaagc cctgaaccca caacacatct gtctccatgg taacagctgc 540
accgg 545

<210> 11
<211> 196
<212> DNA
<213> Homo sapien

<400> 11
tctcctaggc tgggcacagt ggctcatacc tgtaatcctg accgtttcag aggctcaggt 60
ggggggatcg cttgagccca agatttcaag actagtctgg gtaacatagt gagaccctat 120
ctctacgaaa aaataaaaaa atgagcctgg tgtagtggca cacaccagct gaggagggag 180
aatcgagcct aggaga 196

<210> 12
<211> 388
<212> DNA

<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(388)
<223> n = A,T,C or G

<400> 12
tctcctaggc ttgggggctc tgactagaaa ttcaaggaac ctgggattca agtccaactg 60
tgacaccaac ttacactgtg gnctccaata aactgcttct ttcctattcc ctctctatta 120
aataaaataa ggaaaacgat gtctgtgtat agccaagtca gntatcctaa aaggagatac 180
taagtgacat taaatatcag aatgtaaaac ctgggaacca ggttcccagc ctgggattaa 240
actgacagca agaagactga acagtactac tgtgaaaagc ccgaagnggc aatatgttca 300
ctctaccgtt gaaggatggc tgggagaatg aatgctctgt cccccagtcc caagctcact 360
tactatacct cctttatagc ctaggaga 388

<210> 13
<211> 337
<212> DNA
<213> Homo sapien

<400> 13
tagtagttgc ctataatcat gtttctcatt attttcacat tttattaacc aatttctgtt 60
taccctgaaa aatatgaggg aaatatatga aacagggagg caatgttcag ataattgatc 120
acaagatatg atttctacat cagatgctct ttcctttcct gtttatttcc tttttatttc 180
ggttgtgggg tcgaatgtaa tagctttgtt tcaagagaga gttttggcag tttctgtagc 240
ttctgacact gctcatgtct ccaggcatct atttgcactt taggaggtgt cgtgggagac 300
tgagaggtct atttttttcca tatttgggca actacta 337

<210> 14
<211> 571
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(571)
<223> n = A,T,C or G

<400> 14
tagtagttgc catacagtgc ctttccattt atttaacccc cacctgaacg gcataaactg 60
agtgttcagc tggtgttttt tactgtaaac aataaggaga ctttgctctt catttaaacc 120
aaaatcatat ttcatatttt acgctcgagg gtttttaccg gttccttttt acactcctta 180
aaacagtttt taagtcgttt ggaacaagat attttttctt tcctggcagc ttttaacatt 240
atagcaaatt tgtgtctggg ggactgctgg tcactgtttc tcacagttgc aaatcaaggc 300
atttgcaacc aagaaaaaaa aatttttttg ttttatttga aactggaccg gataaacggt 360
gtttggagcg gctgctgtat atagttttaa atggtttatt gcacctcctt aagttgcact 420
tatgtggggg ggggnttttg natagaaagt ntttantcac anagtcacag ggacttttnt 480
cttttggnna ctgagctaaa aagggctgnt tttcgggtgg gggcagatga aggctcacag 540
gaggcctttc tcttagaggg gggaactnct a 571

<210> 15
<211> 548
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(548)
<223> n = A,T,C or G

<400> 15
tatatattta ataacttaaa tatattttga tcacccactg gggtgataag acaatagata 60
taaaagtatt tccaaaaagc ataaaaccaa agtatcatac caaaccaaat tcatactgct 120
tcccccaccc gcactgaaac ttcaccttct aactgtctac ctaaccaaat tctacccttc 180
aagtctttgg tgcgtgctca ctactctttt ttttttttt tttnttttgg agatggagtc 240
tggctgtgca gcccaggggt ggagtacaat ggcacaacct cagctcactg naacctccgc 300
ctcccaggtt catgagattc tcctgnttca gccttcccag tagctgggac tacaggtgtg 360
catcaccatg cctggntaat cttttttngt tttngggtag agatgggggt tttacatgtt 420
ggccaggntg gtntcgaact cctgacctca agtgatccac ccacctcagg ctcccaaagt 480
gctaggatta cagacatgag ccactgngcc cagncctggt gcatgctcac ttctctaggc 540
aactacta 548

<210> 16
<211> 638
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(638)
<223> n = A,T,C or G

<400> 16
ttccgttatg cacatgcaga atattctatc ggtacttcag ctattactca ttttgatggc 60
gcaatccgag cctatcctca agatgagtat ttagaaagaa ttgatttagc gatagaccaa 120
gctggtaagc actctgacta cacgaaattg ttcagatgtg atggatttat gacagttgat 180
ctttggaaga gattattaag tgattatttt aaagggaatc cattaattcc agaatatctt 240
ggtttagctc aagatgatat agaaatagaa cagaaagaga ctacaaatga agatgtatca 300
ccaactgata ttgaagagcc tatagtagaa aatgaattag ctgcatttat tagccttaca 360
catagcgatt ttcctgatga atcttatatt cagccatcga catagcatta cctgatgggc 420
aaccttacga ataatagaaa ctgggtgcgg ggctattgat gaattcatcc ncagtaaatt 480
tggatatnac aaaatataac tcgattgcat ttggatgatg gaatactaaa tctggcaaaa 540
gtaactttgg agctactagt aacctctctt tttgagatgc aaaattttct tttagggttt 600
cttattctct actttacgga tattggagca taacggga 638

<210> 17
<211> 286
<212> DNA
<213> Homo sapien

<400> 17

actgatggat gtcgccggag gcgaggggcc ttatctgatg ctcggctgcc tgttcgtgat 60
gtgcgcggcg attgggctgt ttatctcaaa caccgccacg gcggtgctga tggcgcctat 120
tgccttagcg gcggcgaagt caatgggcgt ctcaccctat ccttttgcca tggtggtggc 180
gatggcggct tcggcggcgt ttatgacccc ggtctcctcg ccggttaaca ccctggtgct 240
tggccctggc aagtactcat ttagcgattt tgtcaaaata ggcgtg 286

<210> 18
<211> 262
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(262)
<223> n = A,T,C or G

<400> 18
tcggtcatag cagccccttc ttctcaattt catctgtcac taccctggtg tagtatctca 60
tagccttaca tttttatagc ctcctccctg gtctgtcttt tgattttcct gcctgtaatc 120
catatcacac ataactgcaa gtaaacattt ctaaagtgtg gttatgctca tgtcactcct 180
gtgncaagaa atagtttcca ttaccgtctt aataaaattc ggatttgttc tttnctattn 240
tcactcttca cctatgaccg aa 262

<210> 19
<211> 261
<212> DNA
<213> Homo sapien

<400> 19
tcggtcatag caaagccagt ggtttgagct ctctactgtg taaactccta aaccaaggcc 60
atttatgata aatggtggca ggatttttat tataaacatg tacccatgca aatttcctat 120
aactctgaga tatattcttc tacatttaaa caataaaaat aatctatttt taaaagccta 180
atttgcgtag ttaggtaaga gtgtttaatg agagggtata aggtataaat caccagtcaa 240
cgtttctctg cctatgaccg a 261

<210> 20
<211> 294
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(294)
<223> n = A,T,C or G

<400> 20
tacaacgagg cgacgtcggt aaaatcggac atgaagccac cgctggtctt ttcgtccgag 60
cgataggcgc cggccagcca gcggaacggt tgcccggatg gcgaagcgag ccggagttct 120
tcggactgag tatgaatctt gttgtgaaaa tactcgccgc cttcgttcga cgacgtcgcg 180
tcgaaatctt cganctcctt acgatcgaag tcttcgtggg cgacgatcgc ggtcagttcc 240
gccccaccga aatcatggtt gagccggatg ctgnccccga agncctcgtt tgtn 294

<210> 21
<211> 208
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(208)
<223> n = A,T,C or G

<400> 21
ttggtaaagg gcatggacgc agacgcctga cgtttggctg aaaatctttc attgattcgt 60
atcaatgaat aggaaaattc ccaaagaggg aatgtcctgt tgctcgccag tttttntgtt 120
gttctcatgg anaaggcaan gagctcttca gactattggn attntcgttc ggtcttctgc 180
caactagtcg ncttgcnang atcttcat 208

<210> 22
<211> 287
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(287)
<223> n = A,T,C or G

<400> 22
nccnttgagc tgagtgattg agatntgtaa tggttgtaag ggtgattcag gcggattagg 60
gtggcgggtc acccggcagt gggtctcccg acaggccagc aggatttggg gcaggtacgg 120
ngtgcgcatc gctcgactat atgctatggc aggcgagccg tggaaggngg atcaggtcac 180
ggcgctggag ctttccacgg tccatgnatt gngatggctg ttctaggcgg ctgttgccaa 240
gcgtgatggt acgctggctg gagcattgat ttctggtgcc aaggtgg 287

<210> 23
<211> 204
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(204)
<223> n = A,T,C or G

<400> 23
ttgggtaaag ggagcaagga gaaggcatgg agaggctcan gctggtcctg gcctacgact 60
gggccaagct gtcgccgggg atggtggaga actgaagcgg gacctcctcg aggtcctccg 120
ncgttacttc nccgtccagg aggagggtct ttccgtggtc tnggaggagc ggggggagaa 180
gatnctcctc atggtcnaca tccc 204

<210> 24

<211> 264
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(264)
<223> n = A,T,C or G

<400> 24
tggattggtc aggagcgggt agagtggcac cattgagggg atattcaaaa atattatttt 60
gtcctaaatg atagttgctg agtttttctt tgacccatga gttatattgg agtttatttt 120
ttaactttcc aatcgcatgg acatgttaga cttattttct gttaatgatt nctattttta 180
ttaaattgga tttgagaaat tggttnttat tatatcaatt tttggtattt gttgagtttg 240
acattatagc ttagtatgtg acca 264

<210> 25
<211> 376
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(376)
<223> n = A,T,C or G

<400> 25
ttacaacgag ggaaactcc gtctctacaa aaattaaaaa attagccagg tgtggtggtg 60
tgcacccgca atcccagcta cttgggaggt tgagacacaa gantcaccta natgtgggag 120
gtcaaggttg catgagtcat gattgtgcca ctgcactcca gcctgggtga cagaccgaga 180
ccctgcctca anaganaang aataggaagt tcagaaatcn tggntgtggn gcccagcaat 240
ctgcatctat ncaacccctg caggcaangc tgatgcagcc tangttcaag agctgctgtt 300
tctggaggca gcagttnggg cttccatcca gtatcacggc cacactcgca cnagccatct 360
gtcctccgtn tgtnac 376

<210> 26
<211> 372
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(372)
<223> n = A,T,C or G

<400> 26
ttacaacgag ggaaactcc gtctctacaa aaattaaaaa attagccagg tgtggtggtg 60
tgcacctgta atcccagcta cttgggcggc tgagacacaa gaaccaccta aatgtgggag 120
ggtcaaggtt gcatgagtca tgatcgcgcc actgcactcc agcctgggtg acagactgag 180
accctgcctc aaaagaaaaa gaataggaag ttcagaaacc ctgggtgtgg ngcccagcaa 240
tctgcattta aacaatccct gcaggcaatg ctgatgcagc ctaagttcaa gagctgctgt 300

tctggaggca gnagtaaggg cttccatcca gcatcacggn caacactgca aaagcacctg 360
tcctcgttgg ta 372

<210> 27
<211> 477
<212> DNA
<213> Homo sapien

<400> 27
ttctgtccac atctacaagt tttatttatt ttgtgggttt tcagggtgac taagtttttc 60
cctacattga aaagagaagt tgctaaaagg tgcacaggaa atcatttttt taagtgaata 120
tgataatatg ggtccgtgct taatacaact gagacatatt tgttctctgt ttttttagag 180
tcacctctta aagtccaatc ccacaatggt gaaaaaaaaa tagaaagtat ttgttctacc 240
tttaaggaga ctgcagggat tctccttgaa aacggagtat ggaatcaatc ttaaataaat 300
atgaaattgg ttggtcttct gggataagaa attcccaact cagtgtgctg aaattcacct 360
gacttttttt gggaaaaaat agtcgaaaat gtcaatttgg tccataaaat acatgttact 420
attaaaagat atttaaagac aaattctttc agagctctaa gattggtgtg gacagaa 477

<210> 28
<211> 438
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(438)
<223> n = A,T,C or G

<400> 28
tctncaacct cttgantgtc aaaaaccttn taggctatct ctaaaagctg actggtattc 60
attccagcaa aatccctcta gtttttggag tttccttta ctatctgggg ctgcctgagc 120
cacaaatgcc aaattaagag catggctatt ttcgggggct gacaggtcaa aaggggtgta 180
aatccgataa gcctcctgga ggtgctctaa aaacactcct ggtgactcat catgcccctg 240
gacgacttca atcgncttag acaagtttat aggtttctgg gcagctccct gaatacccac 300
gaggagatac cggtggaaat cgtcaaaagt tctccctcca cttgagaaat ttgggtccca 360
attaggtccc aattgggtct ctaatcacta ttcctctagc ttcctcctcc ggnctattgg 420
ttgatgtgag gttgaaga 438

<210> 29
<211> 620
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(620)
<223> n = A,T,C or G

<400> 29
aagagggtac cagccccaag ccttgacaac ttccataggg tgtcaagcct gtgggtgcac 60
agaagtcaaa aattgagttt tgggatcctc agcctagatt tcagaggata taaagaaaca 120

cctaacacct agatattcag acaaaagttt actacaggga tgaagctttc acggaaaacc 180
tctactagga aagtacagaa gagaaatgtg ggtttggagc ccccaaacag aatcccctct 240
agaacactgc ctaatgaaac tgtgagaaga tggccactgt catccagaca ccagaatgat 300
agacccacca aaaacttatg ccatattgcc tataaaacct acagacactc aatgccagcc 360
ccatgaaaaa aaaactgaga agaagactgt nccctacaat gccaccggag cagaactgcc 420
ccaggccatg gaagcacagc tcttatatca atgtgacctg gatgttgaga catggaatcc 480
nangaaatcn ttttaanact tccacggttn aatgactgcc ctattanatt cngaacttan 540
atccnggcct gtgacctctt tgctttggcc attccccctt tttggaatgg ctnttttttt 600
cccatgcctg tnccctctta 620

<210> 30
<211> 100
<212> DNA
<213> Homo sapien

<400> 30
ttacaacgag ggggtcaatg tcataaatgt cacaataaaa caatctcttc tttttttttt 60
tttttttttt tttttttttt tttttttttt tttttttttt 100

<210> 31
<211> 762
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(762)
<223> n = A,T,C or G

<400> 31
tagtctatgc gccggacaga gcagaattaa attggaagtt gccctccgga ctttctaccc 60
acactcttcc tgaaaagaga aagaaaagag gcaggaaaga ggttaggatt tcattttcaa 120
gagtcagcta attaggagag cagagtttag acagcagtag gcaccccatg atacaaacca 180
tggacaaagt ccctgtttag taactgccag acatgatcct gctcaggttt tgaaatctct 240
ctgcccataa aagatggaga gcaggagtgc catccacatc aacacgtgtc caagaaagag 300
tctcagggag acaagggtat caaaaaacaa gattcttaat gggaaggaaa tcaaaccaaa 360
aaattagatt tttctctaca tatatataat atacagatat ttaacacatt attccagagg 420
tggctccagt ccttggggct tgagagatgg tgaaaacttt tgttccacat taacttctgc 480
tctcaaattc tgaagtatat cagaatggga caggcaatgt tttgctccac actggggcac 540
agacccaaat ggttctgtgc ccgaagaaga gaagcccgaa agacatgaag gatgcttaag 600
gggggttggg aaagccaaat tggtantatc ttttcctcct gcctgtgttc cngaagtctc 660
cnctgaagga attcttaaaa ccctttgtga ggaaatgccc ccttaccatg acaantggtc 720
ccattgcttt tagggngatg gaaacaccaa gggttttgat cc 762

<210> 32
<211> 276
<212> DNA
<213> Homo sapien

<400> 32
tagtctatgc gtgtattaac ctccccctccc tcagtaacaa ccaaagaggc aggagctgtt 60

attaccaacc ccattttaca gatgcatcaa taatgacaga gaagtgaagt gacttgcgca 120
cacaaccagt aaattggcag agtcagattt gaatccatgg agtctggtct gcactttcaa 180
tcaccgaata ccctttctaa gaaacgtgtg ctgaatgagt gcatggataa atcagtgtct 240
actcaacatc tttgcctaga tatcccgcat agacta 276

<210> 33
<211> 477
<212> DNA
<213> Homo sapien

<400> 33
tagtagttgc caaatatttg aaaatttacc cagaagtgat tgaaaacttt ttggaaacaa 60
aaacaaataa agccaaaagg taaaataaaa atatctttgc actctcgtta ttacctatcc 120
ataacttttt caccgtaagc tctcctgctt gttagtgtag tgtggttata ttaaactttt 180
tagttattat tttttattca cttttccact agaaagtcat tattgattta gcacacatgt 240
tgatctcatt tcatttttc tttttatagg caaaatttga tgctatgcaa caaaaatact 300
caagcccatt atctttttc cccccgaaat ctgaaaattg caggggacag agggaagtta 360
tcccattaaa aaattgtaaa tatgttcagt ttatgtttaa aaatgcacaa aacataagaa 420
aattgtgttt acttgagctg ctgattgtaa gcagttttat ctcaggggca actacta 477

<210> 34
<211> 631
<212> DNA
<213> Homo sapien

<400> 34
tagtagttgc caattcagat gatcagaaat gctgctttcc tcagcattgt cttgttaaac 60
cgcatgccat ttggaacttt ggcagtgaga agccaaaagg aagaggtgaa tgacatatat 120
atatatatat attcaatgaa agtaaaatgt atatgctcat atactttcta gttatcagaa 180
tgagttaagc tttatgccat tgggctgctg catattttaa tcagaagata aaagaaaatc 240
tgggcatttt tagaatgtga tacatgtttt tttaaaactg ttaaatatta tttcgatatt 300
tgtctaagaa ccggaatgtt cttaaaattt actaaaacag tattgtttga ggaagagaaa 360
actgtactgt ttgccattat tacagtcgta caagtgcatg tcaagtcacc cactctctca 420
ggcatcagta tccacctcat agctttacac attttgacgg ggaatattgc agcatcctca 480
ggcctgacat ctgggaaagg ctcagatcca cctactgctc cttgctcgtt gatttgtttt 540
aaaatattgt gcctggtgtc acttttaagc cacagccctg cctaaaagcc agcagagaac 600
agaacccgca ccattctata ggcaactact a 631

<210> 35
<211> 578
<212> DNA
<213> Homo sapien

<400> 35
tagtagttgc catcccatat tacagaaggc tctgtataca tgacttattt ggaagtgatc 60
tgttttctct ccaaacccat ttatcgtaat ttcaccagtc ttggatcaat cttggtttcc 120
actgatacca tgaaacctac ttggagcaga cattgcacag ttttctgtgg taaaaactaa 180
aggtttattt gctaagctgt catcttatgc ttagtatttt ttttttacag tggggaattg 240
ctgagattac attttgttat tcattagata ctttgggata acttgacact gtcttctttt 300
tttcgctttt aattgctatc atcatgcttt tgaaacaaga acacattagt cctcaagtat 360
tacataagct tgcttgttac gcctggtggt ttaaaggact atctttggcc tcaggttcac 420

aagaatgggc aaagtgtttc cttatgttct gtagttctca ataaaagatt gccaggggcc 480
gggtactgtg gctcgcactg taatcccagc actttgggaa gctgaggctg gcggatcatg 540
ttagggcagg tgttcgaaac cagcctgggc aactacta 578

<210> 36
<211> 583
<212> DNA
<213> Homo sapien

<400> 36
tagtagttgc ctgtaatccc agcaactcag gaggctgggg caggagaatc agttgaacct 60
gggaggcaga agttgtaatt agcaaagatc gcaccattgc acttcagcct gggcaacaag 120
agtgagattc catctcaaaa acaaaaaaaa gaaaaagaaa agaaaaggaa aaaacgtata 180
aacccagcca aaacaaaatg atcattcttt taataagcaa gactaattta atgtgtttat 240
ttaatcaaag cagttgaatc ttctgagtta ttggtgaaaa tacccatgta gttaatttag 300
ggttcttact tgggtgaacg tttgatgttc acaggttata aaatggttaa caaggaaaat 360
gatgcataaa gaatcttata aactactaaa aataaataaa atataaatgg ataggtgcta 420
tggatggagt ttttgtgtaa tttaaaatct tgaagtcatt ttggatgctc attggttgtc 480
tggtaatttc cattaggaaa aggttatgat atggggaaac tgtttctgga aattgcggaa 540
tgtttctcat ctgtaaaatg ctagtatctc agggcaacta cta 583

<210> 37
<211> 716
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(716)
<223> n = A,T,C or G

<400> 37
gatctactag tcatntggat tctatccatg gcagctaagc ctttctgaat ggattctact 60
gctttcttgt tctttaatcc agacccttat atatgtttat gttcacaggc agggcaatgt 120
ttagtgaaaa caattctaaa ttttttattt tgcattttca tgctaatttc cgtcacactc 180
cagcaggctt cctgggagaa taaggagaaa tacagctaaa gacattgtcc ctgcttactt 240
acagcctaat ggtatgcaaa accacttcaa taaagtaaca ggaaaagtac taaccaggta 300
gaatggacca aaactgatat agaaaaatca gaggaagaga ggaacaaata tttactgagt 360
cctagaatgt acaaggcttt ttaattacat attttatgta aggcctgcaa aaaacaggtg 420
agtaatcaac atttgtccca ttttacatat aaggaaactg aagcttaaat tgaataattt 480
aatgcataga ttttatagtt agaccatgtt caggtcccta tgttatactt actagctgta 540
tgaatatgag aaaataattt tgttattttc ttggcatcag tattttcatc tgcaaaataa 600
agctaaagtt atttagcaaa cagtcagcat agtgcctgat acatagtagg tgctccaaac 660
atgattacnc tantattngg tattanaaaa atccaatata ggcntggata aaaccg 716

<210> 38
<211> 688
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(688)
<223> n = A,T,C or G

<400> 38
ttctgtccac atatcatccc actttaattg ttaatcagca aaactttcaa tgaaaaatca 60
tccattttaa ccaggatcac accaggaaac tgaaggtgta ttttttttta ccttaaaaaa 120
aaaaaaaaaa accaaacaaa ccaaaacaga ttaacagcaa agagttctaa aaaatttaca 180
tttctcttac aactgtcatt cagagaacaa tagttcttaa gtctgttaaa tcttggcatt 240
aacagagaaa cttgatgaan agttgtactt ggaatattgt ggatttttt ttttgtctaa 300
tctcccccta ttgttttgcc aacagtaatt taagtttgtg tggaacatcc ccgtagttga 360
agtgtaaaca atgtatagga aggaatatat gataagatga tgcatcacat atgcattaca 420
tgtagggacc ttcacaactt catgcactca gaaaacatgc ttgaagagga ggagaggacg 480
gcccagggtc accatccagg tgccttgagg acagagaatg cagaagtggc actgttgaaa 540
tttagaagac catgtgtgaa tggtttcagg cctgggatgt ttgccaccaa gaagtgcctc 600
cgagaaattt ctttcccatt tggaatacag ggtggcttga tgggtacggt gggtgaccca 660
acgaagaaaa tgaaattctg ccctttcc 688

<210> 39
<211> 585
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(585)
<223> n = A,T,C or G

<400> 39
tagtagttgc cgcnnaccta aaanttggaa agcatgatgt ctaggaaaca tantaaaata 60
gggtatgcct atgtgctaca gagagatgtt agcatttaaa gtgcatantt ttatgtattt 120
tgacaaatgc atatncctct ataatccaca actgattacg aagctattac aattaaaaag 180
tttggccggg cgtggtgggc ggtggctgac gcctgtaatc ccagcacttt gggaggccga 240
ggcacgcgga tcacgaggtc gggagttcaa gaccatcctg gctaacacgg tgaaagtcca 300
tctctactaa aaatacgaaa aaattacccc ggcgtggtgg cgggcgcctg tagtcccagc 360
tactccggag gctgaggcag gagaatggcg tgaacccagg acacggagct tgcagtgtgc 420
caacatcacg tcactgccct ccagcctggg ggacaggaac aagantcccg tcctcanaaa 480
agaaaaatac tactnatant ttcnacttta ttttaantta cacagaactn cctcttggta 540
ccccccttacc attcatctca cccacctcct atagggcacn nctaa 585

<210> 40
<211> 475
<212> DNA
<213> Homo sapien

<400> 40
tctgtccaca ccaatcttag aagctctgaa aagaatttgt ctttaaatat cttttaatag 60
taacatgtat tttatggacc aaattgacat tttcgactgt tttttccaaa aaagtcaggt 120
gaatttcagc acactgagtt gggaatttct tatcccagaa gaccaaccaa tttcatattt 180
atttaagatt gattccatac tccgttttca aggagaatcc ctgcagtctc cttaaaggta 240
gaacaaatac ttcctatttt tttttcacca ttgtgggatt ggactttaag aggtgactct 300

aaaaaaacag agaacaaata tgtctcagtt gtattaagca cggacccata ttatcatatt 360
cacttaaaaa aatgatttcc tgtgcacctt ttggcaactt ctcttttcaa tgtagggaaa 420
aacttagtca ccctgaaaac ccacaaaata aataaaactt gtagatgtgg acaga 475

<210> 41
<211> 423
<212> DNA
<213> Homo sapien

<400> 41
taagagggta catcgggtaa gaacgtaggc acatctagag cttagagaag tctggggtag 60
gaaaaaaatc taagtattta taagggtata ggtaacattt aaaagtaggg ctagctgaca 120
ttatttagaa agaacacata cggagagata agggcaaagg actaagacca gaggaacact 180
aatatttagt gatcacttcc attcttggta aaaatagtaa cttttaagtt agcttcaagg 240
aagatttttg gccatgatta gttgtcaaaa gttagttctc ttgggtttat attactaatt 300
ttgttttaag atccttgtta gtgctttaat aaagtcatgt tatatcaaac gctctaaaac 360
attgtagcat gttaaatgtc acaatatact taccatttgt tgtatatggc tgtaccctct 420
cta 423

<210> 42
<211> 527
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(527)
<223> n = A,T,C or G

<400> 42
tctcctaggc taatgtgtgt gtttctgtaa aagtaaaaag ttaaaaattt taaaaataga 60
aaaaagctta tagaataaga atatgaagaa agaaaatatt tttgtacatt tgcacaatga 120
gtttatgttt taagctaagt gttattacaa aagagccaaa aaggttttaa aaattaaaac 180
gtttgtaaag ttacagtacc cttatgttaa tttataattg aagaaagaaa aacttttttt 240
tataaatgta gtgtagccta agcatacagt atttataaag tctggcagtg ttcaataatg 300
tcctaggcct tcacattcac tcactgactc acccagagca acttccagtc ctgtaagctc 360
cattcgtggt aagtgcccta tacaggtgca ccatttattt tacagtattt ttactgtacc 420
ttctctatgt ttccatatgt ttcgatatac aaataccact ggttactatn gcccnacagg 480
taattccagt aacacggcct gtatacgtct ggtancccta gngaaga 527

<210> 43
<211> 331
<212> DNA
<213> Homo sapien

<400> 43
tcttcaacct cgtaggacaa ctctcatatg cctgggcact atttttaggt tactaccttg 60
gctgcccttc tttaagaaaa aaaaaagaag aaaaaagaac ttttccacaa gtttctcttc 120
ctctagttgg aaaattagag aaatcatgtt tttaattttg tgttatttca gatcacaaat 180
tcaaacactt gtaaacatta agcttctgtt caatcccctg ggaagaggat tcattctgat 240
atttacggtt caaaagaagt tgtaatattg tgcttggaac acagagaacc agttattaac 300

ttcctactac tattatataa taaataataa c 331

<210> 44
<211> 592
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(592)
<223> n = A,T,C or G

<400> 44
ggcttagtag ttgccaggca aaatarcgtt gattctcctc aggagccacc cccaacaccc 60
ctgtttgctt ctagacctat acctagacta aagtcccagc agacccctag aggtgaggtt 120
cagagtgacc cttgaggaga tgtgctacac tagaaaagaa ctgcttgagt tttctaattt 180
atataagcag aaatctggag aagagtcata ggaatggata ttaagggtgt gagataatgg 240
cggaaggaat atagagttgg atcaggctgg acttattgat ttgaacccac taagtagaga 300
ttctgctttt gatgttgcag ctcagggagt taaaaaaggt tttaatggtt ctaatagttt 360
atttgcttgg ttagctgaaa tatggataaa agatggccca ctgtgagcaa gctggaaatg 420
cctgatctct ctcagtttaa tgtagaggaa gggatccaaa agtttaggga ganttggatg 480
ctggraktgg attggtcact ttgrgaccta cccwtcccag ctgggagggt ccagaagata 540
caccccttgac caacgctttg cgaaatggat ttgtgatggc ggcaactact aa 592

<210> 45
<211> 567
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(567)
<223> n = A,T,C or G

<400> 45
ggcttagtag ttgccattgc gagtgcttgc tcaacgagcg ttgaacatgg cggattgtct 60
agattcaacg gatttgagtt ttaccagcaa agcgaaccaa gcgcggccca gagaattatg 120
ggttggttgg ctttgaaaag atggaaatcc tgtaggccta gtcagaaaag ccttcttgca 180
gaacagttgg ttctcgggcg aacgctcatc aagatgccca ttggaaaggc tagcgtgtat 240
ttgggagagc ctgatagcgt gtcttctgat gatgtttgtg cttggacagt gacaaaagat 300
atgcaaagca agtccgaact agacgtcaag cttcgtgagc aaattattgt agactcctac 360
ttatactgtg aggaatgata gccaagggtg gggactttaa gactaaggtg gtttgtactt 420
gcgccgatga tcccaggcag aaagamctga tcgctagttt tatacgggca actactaagc 480
cgaattccag cacactggcg gccgttacta attggatccg anctcggtac cagcttgatg 540
catascttga gttwtctata ntgtcnc 567

<210> 46
<211> 908
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(908)
<223> n = A,T,C or G

<400> 46
gagcgaaaga ccgagggcag ngnntangng cgangaagcg gagagggcca aaaagcaacc 60
gctttccccg gggggtgccg attcattaag gcaggtggag gacaggtttc ccgatggaag 120
gcggcagggg cgcaagcaat taatgtgagt aggccattca ttagcacccg ggcttaacat 180
ttaagcttcg ggttggtatg tggtgggaat tgtgagcgga taacaatttc acacaggaaa 240
cagctatgac catgattacg ccaagctatt taggtgacat tatagaataa ctcaagttat 300
gcatcaagct tggtaccgag ttcggatcca ctagtaacgg ccgccagtgt gtggaattcg 360
gcttagtagt tgccgaccat ggagtgctac ctaggctaga atacctgagy tcctccctag 420
cctcactcac attaaattgt atcttttcta cattagatgt cctcagcgcc ttatttctgc 480
tggacwatcg ataaattaat cctgatagga tgatagcagc agattaatta ctgagagtat 540
gttaatgtgt catccctcct atataacgta tttgcatttt aatggagcaa ttctggagat 600
aatccctgaa ggcaaaggaa tgaatcttga gggtgagaaa gccagaatca gtgtccagct 660
gcagttgtgg gagaaggtga tattatgtat gtctcagaag tgacaccata tgggcaacta 720
ctaagcccga attccagcac actggcgggc gttactaatg gatccgagct cggtaccaag 780
cttgatgcat agcttgagta tctatagtgt cactaaatag cctggcgtta tcatggtcat 840
agctgtttcc tgtgtgaaat tgttatccgc tcccaattcc ccccaccata cgagccggaa 900
cataaagt 908

<210> 47
<211> 480
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(480)
<223> n = A,T,C or G

<400> 47
tgccaacaag gaaagtttta aatttcccct tgaggattct tggtgatcat caaattcagt 60
ggtttttaag gttgttttct gtcaaataac tctaacttta agccaaacag tatatggaag 120
cacagataka atattacaca gataaaagag gagttgatct aaagtaraga tagttggggg 180
ctttaatttc tggaacctag gtctccccat cttcttctgt gctgaggaac ttcttggaag 240
cggggattct aaagttcttt ggaagacagt ttgaaaacca ccatgttgtt ctcagtacct 300
ttatttttaa aaagtaggtg aacattttga gagagaaaag ggcttggttg agatgaagtc 360
ccccccccc ctttttttt ttttagctga aatagatacc ctatgttnaa rgaarggatt 420
attatttacc atgccaytar scacatgctc tttgatgggc nyctccstac cctccttaag 480

<210> 48
<211> 591
<212> DNA
<213> Homo sapien

<400> 48
aagagggtac cgagtggaat ttccgcttca ctagtctggt gtggctagtc ggtttcgtgg 60
tggccaacat tacgaacttc caactcaacc gttcttggac gttcaagcgg gagtaccggc 120

gaggatggtg gcgtgaattc tggcctttct ttgccgtggg atcggtagcc gccatcatcg 180
gtatgtttat caagatcttc tttactaacc cgacctctcc gatttacctg cccgagccgt 240
ggtttaacga ggggaggggg atccagtcac gcgagtactg gtcccagatc ttcgccatcg 300
tcgtgacaat gcctatcaac ttcgtcgtca ataagttgtg gaccttccga acggtgaagc 360
actccgaaaa cgtccggtgg ctgctgtgcg gtgactccca aaatcttgat aacaacaagg 420
taaccgaatc gcgctaagga accccggcat ctcgggtact ctgcatatgc gtacccctta 480
agccgaattc cagcacactg gcggccgtta ctaattggat ccgaactccg taaccaagcc 540
tgatgcgtaa cttgagttat tctatagtgt ccctaaaata acctggcgtt a 591

<210> 49
<211> 454
<212> DNA
<213> Homo sapien

<400> 49
aagagggtac ctgccttgaa atttaaatgt ctaaggaaar tgggagatga ttaagagttg 60
gtgtggcyta gtcacaccaa aatgtattta ttacatcctg ctcctttcta gttgacagga 120
aagaaagctg ctgtggggaa aggagggata aatactgaag ggatttacta aacaaatgtc 180
catcacagag ttttcctttt tttttttttg agacagagtc ttgctctgtc acccaggctg 240
gaatgaagwg gtatgatctc agttgaatgc aacctctacc tcctaggttc aagcgattct 300
catgcctcag cctcctgagc agctgggact ataggcgcat gctaccatgc caggctaatt 360
tttatatttt tattagagac ggggtgttgc catgttggcc aggcaggtct cgaactcctg 420
ggcctcagat gatctgcccc accgtaccct ctta 454

<210> 50
<211> 463
<212> DNA
<213> Homo sapien

<400> 50
aagagggtac caaaaaaaag aaaaaggaaa aaaagaaaaa caacttgtat aaggctttct 60
gctgcataca gctttttttt tttaaataaa tggtgccaac aaatgttttt gcattcacac 120
caattgctgg ttttgaaatc gtactcttca aaggtatttg tgcagatcaa tccaatagtg 180
atgccccgta ggttttgtgg actgcccacg ttgtctacct tctcatgtag gagccattga 240
gagactgttt ggacatgcct gtgttcatgt agccgtgatg tccgggggcc gtgtacatca 300
tgttaccgtg gggtggggtc tgcattggct gctgggcata tggctgggtg cccatcatgc 360
ccatctgcat ctgcataggg tattggggcg tttgatccat atagccatga ttgctgtggt 420
agccactgtt catcattggc tgggacatgc tgttaccctc tta 463

<210> 51
<211> 399
<212> DNA
<213> Homo sapien

<400> 51
cttcaacctc ccaaagtgct gggattacag gactgagcca ccacgctcag cctaagcctc 60
tttttcacta ccctctaagc gatctaccac agtgatgagg ggctaaagag cagtgcaatt 120
tgattacaat aatggaactt agatttatta attaacaatt tttccttagc atgttggttc 180
cataattatt aagagtatgg acttacttag aaatgagctt tcattttaag aatttcatct 240
ttgaccttct ctattagtct gagcagtatg acactatacg tattttattt aactaaccta 300
ccttgagcta ttacttttta aaaggctata tacatgaatg tgtattgtca actgtaaagc 360

cccacagtat ttaattatat catgatgtct ttgaggttg 399

<210> 52
<211> 392
<212> DNA
<213> Homo sapien

<400> 52
cttcaacctc aatcaacctt ggtaattgat aaaatcatca cttaactttc tgatataatg 60
gcaataatta tctgagaaaa aaaagtggtg aaagattaaa cttgcatttc tctcagaatc 120
ttgaaggata tttgaataat tcaaaagcgg aatcagtagt atcagccgaa gaaactcact 180
tagctagaac gttggaccca tggatctaag tccctgccct tccactaacc agctgattgg 240
ttttgtgtaa acctcctaca cgcttgggct tggtcgcctc atttgtcaaa gtaaaggctg 300
aaataggaag ataatgaacc gtgtcttttt ggtctctttt ccatccatta ctctgatttt 360
acaaagaggc ctgtattccc ctggtgaggt tg 392

<210> 53
<211> 179
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(179)
<223> n = A,T,C or G

<400> 53
ttcgggtgat gcctcctcag gctacagtga agactggatt acagaaaggt gccagcgaga 60
tttcagattc ctgtaaacct ctaaagaaaa ggagtcgcgc ctcaactgat gtagaaatga 120
ctagttcagc atacngagac acntctgact ccgattctag aggactgagt gacctgcan 179

<210> 54
<211> 112
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(112)
<223> n = A,T,C or G

<400> 54
ttcgggtgat gcctcctcag gctacatcat natagaagca aagtagaana atcnngtttg 60
tgcattttcc cacanacaaa attcaaatga ntggaagaaa ttggganagt at 112

<210> 55
<211> 225
<212> DNA
<213> Homo sapien

<400> 55

tgagcttccg cttctgacaa ctcaatagat aatcaaagga caactttaac agggattcac 60
aaaggagtat atccaaatgc caataaacat ataaaaagga attcagcttc atcatcatca 120
gaagwatgca aattaaaacc ataatgagaa accactatgt cccactagaa tagataaaat 180
cttaaaagac tggtaaaacc aagtgttggt aaggcaagag gagca 225

<210> 56
<211> 175
<212> DNA
<213> Homo sapien

<400> 56
gctcctcttg ccttaccaac acattctcaa aaacctgtta gagtcctaag cattctcctg 60
ttagtattgg gattttaccc ctgtcctata aagatgttat gtaccaaaaa tgaagtggag 120
ggccataccc tgagggaggg gagggatctc tagtgttgtc agaagcggaa gctca 175

<210> 57
<211> 223
<212> DNA
<213> Homo sapien

<400> 57
agccatttac cacccatgga tgaatggatt ttgtaattct agctgttgta ttttgtgaat 60
ttgttaattt tgttgttttt ctgtgaaaca catacattgg atatgggagg taaaggagtg 120
tcccagttgc tcctggtcac tccctttata gccattactg tcttgtttct tgtaactcag 180
gttaggtttt ggtctctctt gctccactgc aaaaaaaaaa aaa 223

<210> 58
<211> 211
<212> DNA
<213> Homo sapien

<400> 58
gttcgaaggt gaacgtgtag gtagcggatc tcacaactgg ggaactgtca aagacgaatt 60
aactgacttg gatcaatcaa atgtgactga ggaaacacct gaaggtgaag aacatcatcc 120
agtggcagac actgaaaata aggagaatga agttgaagag gtaaaagagg agggtccaaa 180
agagatgact ttggatgggt ggtaaatggc t 211

<210> 59
<211> 208
<212> DNA
<213> Homo sapien

<400> 59
gctcctcttg ccttaccaac tttgcaccca tcatcaacca tgtggccagg tttgcagccc 60
aggctgcaca tcaggggact gcctcgcaat acttcatgct gttgctgctg actgatggtg
120ctgtgacgga tgtggaagcc acacgtgagg ctgtggtgcg tgcctcgaac ctgcccatgt 180
cagtgatcat tatgggtggt aaatggct 208

<210> 60
<211> 171
<212> DNA

<213> Homo sapien

<400> 60
agccatttac cacccatact aaattctagt tcaaactcca acttcttcca taaaacatct 60
aaccactgac accagttggc aatagcttct tccttcttta acctcttaga gtatttatgg 120
tcaatgccac acatttctgc aactgaataa agttggtaag gcaagaggag c 171

<210> 61
<211> 134
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(134)
<223> n = A,T,C or G

<400> 61
cgggtgatgc ctcctcaggc tttggtgtgt ccactcnact cactggcctc ttctccagca 60
actggtgaan atgtcctcan gaaaancncc acacgcngct cagggtgggg tgggaancat 120
canaatcatc nggc 134

<210> 62
<211> 145
<212> DNA
<213> Homo sapien

<400> 62
agagggtaca tatgcaacag tatataaagg aagaagtgca ctgagaggaa cttcatcaag 60
gccatttaat caataagtga tagagtcaag gctcaaccca ggtgtgacgg attccaggtc 120
ccaagctcct tactggtacc ctctt 145

<210> 63
<211> 297
<212> DNA
<213> Homo sapien

<400> 63
tgcactgaga ggaattcaaa gggtttatgc caaagaacaa accagtcctc tgcagcctaa 60
ctcatttgtt tttgggctgc gaagccatgt agagggcgat caggcagtag atggtccctc 120
ccacagtcag cgccatggtg gtccggtaaa gcatttggtc aggcaggcct cgtttcaggt 180
agacgggcac acatcagctt tctggaaaaa cttttgtagc tctggagctt tgtttttccc 240
agcataatca tacactgtgg aatcggaggt cagtttagtt ggtaaggcaa gaggagc 297

<210> 64
<211> 300
<212> DNA
<213> Homo sapien

<400> 64
gcactgagag gaacttccaa tactatgttg aataggagtg gtgagagagg gcatccttgt 60

cttgtgccgg ttttcaaagg gaatgcttcc agcttttgcc cattcagtat aatattaaag 120
aatgttttac cattttctgt cttgcctgtt tttctgtgtt tttgttggtc tcttcattct 180
ccatttttag gcctttacat gttaggaata tatttctttt aatgatactt caccttggt 240
atcttttgtg agactctact catagtgtga taagcactgg gttggtaagg caagaggagc 300

<210> 65
<211> 203
<212> DNA
<213> Homo sapien

<400> 65
gctcctcttg ccttaccaac tcacccagta tgtcagcaat tttatcrgct ttacctacga 60
aacagcctgt atccaaacac ttaacacact cacctgaaaa gttcaggcaa caatcgcctt 120
ctcatgggtc tctctgctcc agttctgaac ctttctcttt tcctagaaca tgcatttarg 180
tcgatagaag ttcctctcag tgc 203

<210> 66
<211> 344
<212> DNA
<213> Homo sapien

<400> 66
tacggggacc cctgcattga gaaagcgaga ctcactctga agctgaaatg ctgttgccct 60
tgcagtgctg gtagcaggag ttctgtgctt tgtgggctaa ggctcctgga tgacccctga 120
catggagaag gcagagttgt gtgccccttc tcatggcctc gtcaaggcat catggactgc 180
cacacacaaa atgccgtttt tattaacgac atgaaattga aggagagaac acaattcact 240
gatgtggctc gtaaccatgg atatggtcac atacagaggt gtgattatgt aaaggttaat 300
tccacccacc tcatgtggaa actagcctca atgcaggggt ccca 344

<210> 67
<211> 157
<212> DNA
<213> Homo sapien

<400> 67
gcactgagag gaacttcgta gggaggttga actggctgct gaggaggggg aacaacaggg 60
taaccagact gatagccatt ggatggataa tatggtggtt gaggagggac actacttata 120
gcagagggtt gtgtatagcc tgaggaggca tcacccg 157

<210> 68
<211> 137
<212> DNA
<213> Homo sapien

<400> 68
gcactgagag gaacttctag aaagtgaaag tctagacata aaataaaata aaaatttaaa 60
actcaggaga gacagcccag cacggtggct cacgcctgta atcccagaac tttgggagcc 120
tgaggaggca tcacccg 137

<210> 69
<211> 137

<212> DNA
<213> Homo sapien

<400> 69
cgggtgatgc ctcctcaggc tgtattttga agactatcga ctggacttct tatcaactga 60
agaatccgtt aaaaatacca gttgtattat ttctacctgt caaaatccat ttcaaatgtt 120
gaagttcctc tcagtgc 137

<210> 70
<211> 220
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(220)
<223> n = A,T,C or G

<400> 70
agcatgttga gcccagacac gcaatctgaa tgagtgtgca cctcaagtaa atgtctacac 60
gctgcctggt ctgacatggc acaccatcnc gtggagggca casctctgct cngcctacwa 120
cgagggcant ctcatwgaca ggttccaccc accaaactgc aagaggctca nnaagtactr 180
ccagggtmya sggacmasgg tgggaytyca ycacwcatct 220

<210> 71
<211> 353
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(353)
<223> n = A,T,C or G

<400> 71
cgttagggtc tctatccact gctaaaccat acacctgggt aaacagggac catttaacat 60
tcccanctaa atatgccaag tgacttcaca tgtttatctt aaagatgtcc aaaacgcaac 120
tgattttctc ccctaaacct gtgatggtgg gatgattaan cctgagtggt ctacagcaag 180
ttaagtgcaa ggtgctaaat gaangtgacc tgagatacag catctacaag gcagtacctc 240
tcaacncagg gcaactttgc ttctcanagg gcatttagca gtgtctgaag taatttctgt 300
attacaactc acggggcggg gggtgaatat ctantggana gnagacccta acg 353

<210> 72
<211> 343
<212> DNA
<213> Homo sapien

<400> 72
gcactgagag gaacttccaa tacyatkatc agagtgaaca rgcarccyac agaacaggag 60
aaaatgttyg caatctctcc atctgacaaa aggctaatat ccagawtcta awaggaactt 120
aaacaaattt atgagaaaag aacaracaac ctcawcaaaa agtgggtgaa ggawatgcts 180

aaargaagac atytattcag ccagtaaaca yatgaaaaaa aggctcatsa tcactgawca 240
ttagagaaat gcaaatcaaa accacaatga gataccatct yayrccagtt agaayggtga 300
tcattaaaar stcaggaaac aacagatgct ggacaaggtg tca 343

<210> 73
<211> 321
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(321)
<223> n = A,T,C or G

<400> 73
gcactgagag gaacttcaga gagagagaga gagttccacc ctgtacttgg ggagagaaac 60
agaaggtgag aaagtctttg gttctgaagc agcttctaag atcttttcat ttgcttcatt 120
tcaaagttcc catgctgcca aagtgccatc ctttggggta ctgttttctg agctccagtg 180
ataactcatt tatacaaggg agatacccag aaaaaaagtg agcaaatctt aaaaaggtgg 240
cttgagttca gccttaaata ccatcttgaa atgacacaga gaaagaanga tgttgggtgg 300
gagtggatag agaccctaac g 321

<210> 74
<211> 321
<212> DNA
<213> Homo sapien

<400> 74
gcactgagag gaacttcaga gagagagaga gagttccacc ctgtacttgg ggagagaaac 60
agaaggtgag aaagtctttg gttctgaagc agcttctaag atcttttcat ttgcttcatt 120
tcaaagttcc catgctgcca aagtgccatc ctttggggta ctgttttctg agctccagtg 180
ataactcatt tatacaaggg agatacccag aaaaaaagtg agcaaatctt aaaaaggtgg 240
cttgagttca gycttaaata ccatcttgaa atgamacaga gaaagaagga tgttgggtgg 300
gagtggatag agaccctaac g 321

<210> 75
<211> 317
<212> DNA
<213> Homo sapien

<400> 75
gcactgagag gaacttccac atgcactgag aaatgcatgt tcacaaggac tgaagtctgg 60
aactcagttt ctcagttcca atcctgattc aggtgtttac cagctacaca accttaagca 120
agtcagataa ccttagcttc ctcatatgca aaatgagaat gaaaagtact catcgctgaa 180
ttgttttgag gattagaaaa acatctggca tgcagtagaa attcaattag tattcatttt 240
cattcttcta aattaaacaa ataggatttt tagtggtgga acttcagaca ccagaaatgg 300
gagtggatag agaccct 317

<210> 76
<211> 244
<212> DNA

<213> Homo sapien

<400> 76
cgttagggtc tctatccact cccactactg atcaaactct atttatttaa ttatttttat 60
catactttaa gttctgggat acacgtgcag catgcgcagg tttgttgcat aggtatacac 120
ttgccatggt ggtttgctgc acccatcagt ccatcatcta cattaggtat ttctcctaat 180
gctatccctc ccctagcccc ttacaccccc aacaggctct agtgtgtgaa gttcctctca 240
gtgc 244

<210> 77
<211> 254
<212> DNA
<213> Homo sapien

<400> 77
cgttagggtc tctatccact gaaatctgaa gcacaggagg aagagaagca gtyctagtga 60
gatggcaagt tcwtttacca cactctttaa catttygttt agttttaacc tttatttatg 120
gataataaag gttaatatta ataatgattt attttaaggc attcccraat ttgcataatt 180
ctccttttgg agataccctt ttatctccag tgcaagtctg gatcaaagtg atasamagaa 240
gttcctctca gtgc 254

<210> 78
<211> 355
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(355)
<223> n = A,T,C or G

<400> 78
ttcgatacag gcaaacatga actgcaggag ggtggtgacg atcatgatgt tgccgatggt 60
ccggatggnc acgaagacgc actggancac gtgcttacgt ccttttgctc tgttgatggc 120
cctgagggga cgcaggaccc ttatgaccct cagaatcttc acaacgggag atggcactgg 180
attgantccc antgacacca gagacacccc aaccaccagn atatcantat attgatgtag 240
ttcctgtaga nggccccctt gtggaggaaa gctccatnag ttggtcatct tcaacaggat 300
ctcaacagtt tccgatggct gtgatgggca tagtcatant taaccntgtn tcgaa 355

<210> 79
<211> 406
<212> DNA
<213> Homo sapien

<400> 79
taagagggta ccagcagaaa ggttagtatc atcagatagc atcttatacg agtaatatgc 60
ctgctatttg aagtgtaatt gagaaggaaa attttagcgt gctcactgac ctgcctgtag 120
ccccagtgac agctaggatg tgcattctcc agccatcaag agactgagtc aagttgttcc 180
ttaagtcaga acagcagact cagctctgac attctgattc gaatgacact gttcaggaat 240
cggaatcctg tcgattagac tggacagctt gtggcaagtg aatttgcctg taacaagcca 300
gatttttaa aatttatatt gtaaataatg tgtgtgtgtg tgtgtgtata tatatatata 360

tgtacagtta tctaagttaa tttaaaagtt gtttggtacc ctctta 406

<210> 80
<211> 327
<212> DNA
<213> Homo sapien

<400> 80
ttttttttt tttactcggc tcagtctaat ccttttgta gtcactcata ggccagactt 60
agggctagga tgatgattaa taagagggat gacataacta ttagtggcag gttagttgtt 120
tgtagggctc atggtagggg taaaaggagg gcaatttcta gatcaaataa taagaaggta 180
atagctacta agaagaattt tatggagaaa gggacgcggg cgggggatat agggtcgaag 240
ccgcactcgt aaggggtgga tttttctatg tagccgttga gttgtggtag tcaaaatgta 300
ataattatta gtagtaagcc taggaga 327

<210> 81
<211> 318
<212> DNA
<213> Homo sapien

<400> 81
tagtctatgc ggttgattcg gcaatccatt atttgctgga ttttgtcatg tgttttgcca 60
attgcattca taatttatta tgcatttatg cttgtatctc ctaagtcatg gtatataatc 120
catgcttttt atgttttgtc tgacataaac tcttatcaga gccctttgca cacagggatt 180
caataaatat taacacagtc tacatttatt tggtgaatat tgcatatctg ctgtactgaa 240
agcacattaa gtaacaaagg caagtgagaa gaatgaaaag cactactcac aacagttatc 300
atgattgcgc atagacta 318

<210> 82
<211> 338
<212> DNA
<213> Homo sapien

<400> 82
tcttcaacct ctactcccac taatagcttt ttgatgactt ctagcaagcc tcgctaacct 60
cgccttaccc cccactatta acctactggg agaactctct gtgctagtaa ccacgttctc 120
ctgatcaaat atcactctcc tacttacagg actcaacata ctagtcacag ccctatactc 180
cctctacata tttaccacaa cacaatgggg ctcactcacc caccacatta acaacataaa 240
accctcattc acacgagaaa acaccctcat gttcatacac ctatccccca ttctcctcct 300
atccctcaac cccgacatca ttaccgggtt ttcctctt 338

<210> 83
<211> 111
<212> DNA
<213> Homo sapien

<400> 83
agccatttac cacccatcca caaaaaaaaa aaaaaaaaag aaaaatatca aggaataaaa 60
atagactttg aacaaaaagg aacatttgct ggcctgagga ggcatcaccc g 111

<210> 84

<211> 224
<212> DNA
<213> Homo sapien

<400> 84
tcgggtgatg cctcctcagg ccaagaagat aaagcttcag accccctaaca catttccaaa 60
aaggaagaaa ggagaaaaaa gggcatcatc cccgttccga agggtcaggg aggaggaaat 120
tgaggtggat tcacgagttg cggacaactc ctttgatgcc aagcgaggtg cagccggaga 180
ctggggagag cgagccaatc aggttttgaa gttcctctca gtgc 224

<210> 85
<211> 348
<212> DNA
<213> Homo sapien

<400> 85
gcactgagag gaacttcgtt ggaaacgggt ttttttcatg taaggctaga cagaagaatt 60
ctcagtaact tccttgtgtt gtgtgtattc aactcacasa gttgaacgat cctttacaca 120
gagcagactt gtaacactct twttgtggaa tttgcaagtg gagatttcag scgctttgaa 180
gtsaaaggta gaaaaggaaa tatcttccta taaaaactag acagaatgat tctcagaaac 240
tcctttgtga tgtgtgcgtt caactcacag agtttaacct ttcwtttcat agaagcagtt 300
aggaaacact ctgtttgtaa agtctgcaag tggatagaga ccctaacg 348

<210> 86
<211> 293
<212> DNA
<213> Homo sapien

<400> 86
gcactgagag gaacttcytt gtgwtgtktg yattcaactc acagagttga asswtsmttt 60
acabagwkca ggcttkcaaa cactcttttt gtmgaatytg caagwggaka tttsrrccrc 120
tttgwggycw wysktmgaaw mggrwatatc ttcwyatmra amctagacag aaksattctc 180
akaawstyyy ytgtgawgws tgcrttcaac tcacagagkt kaacmwtyct kytsatrgag 240
cagttwkgaa actctmtttc tttggattct gcaagtggat agagacccta acg 293

<210> 87
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 87
ctcctaggct 10

<210> 88
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 88
agtagttgcc 10

<210> 89
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 89
ttccgttatg c 11

<210> 90
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 90
tggtaaaggg 10

<210> 91
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 91
tcggtcatag 10

<210> 92
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 92
tacaacgagg 10

<210> 93

<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 93
tggattggtc 10

<210> 94
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 94
ctttctaccc 10

<210> 95
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 95
ttttggctcc 10

<210> 96
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 96
ggaaccaatc 10

<210> 97
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 97
tcgatacagg 10

<210> 98
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 98
ggtactaagg 10

<210> 99
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 99
agtctatgcg 10

<210> 100
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 100
ctatccatgg 10

<210> 101
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 101
tctgtccaca 10

<210> 102
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 102
aagagggtac 10

<210> 103
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 103
cttcaacctc 10

<210> 104
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 104
gctcctcttg ccttaccaac 20

<210> 105
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 105
gtaagtcgag cagtgtgatg 20

<210> 106
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 106
gtaagtcgag cagtctgatg 20

<210> 107
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 107
gacttagtgg aaagaatgta 20

<210> 108
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 108
gtaattccgc caaccgtagt 20

<210> 109
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 109
atggttgatc gatagtggaa 20

<210> 110
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 110
acggggaccc ctgcattgag 20

<210> 111
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 111
tattctagac cattcgctac 20

<210> 112
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 112
acataaccac tttagcgttc 20

<210> 113
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 113
cgggtgatgc ctcctcaggc 20

<210> 114
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 114
agcatgttga gcccagacac 20

<210> 115
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 115
gacaccttgt ccagcatctg 20

<210> 116
<211> 20
<212> DNA

<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 116
tacgctgcaa cactgtggag 20

<210> 117
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 117
cgttagggtc tctatccact 20

<210> 118
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 118
agactgactc atgtccccta 20

<210> 119
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 119
tcatcgctcg gtgactcaag 20

<210> 120
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 120
caagattcca taggctgacc 20

<210> 121
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 121
acgtactggt cttgaaggtc 20

<210> 122
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 122
gacgcttggc cacttgacac 20

<210> 123
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 123
gtatcgacgt agtggtctcc 20

<210> 124
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for amplification from breast tumor cDNA

<400> 124
tagtgacatt acgacgctgg 20

<210> 125
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer for amplification from breast tumor cDNA

<400> 125
cgggtgatgc ctcctcaggc 20

<210> 126
<211> 23
<212> DNA
<213> Artificial Sequence

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<210> 138
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<220>
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<210> 139
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<220>
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<210> 140
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ctccagatgg ggaagacttt ttctccaacc agcaggcagg tccccatcca ctcagacacc 300
agcacgtcca ccttctcggg cagcaccacg tcctccacct tctgctggta cacggtgatg 360
atgtcagcaa agccgttctg cangaccagc tgccccgtgt gctgtgccat ctcactggcc 420
tccaccgcgt acaccgctct aggccgcgca tantgtgcac agaanaaatg atgatccagt 480
cccacagccc acgtccaaga ngactttatc cgtcagggat tctttattct gcaggatgac 540
ctgtggtatt aattgttcgt gtctgggctc aacatgcta 579

<210> 148
<211> 249
<212> DNA
<213> Homo sapien

<400> 148
tgacaccttg tccagcatct gcaagccagg aagagagtcc tcaccaagat ccccaccccg 60
ttggcaccag gatcttggac ttccaatctc cagaactgtg agaaataagt atttgtcgct 120
aaataaatct ttgtggtttc agatatttag ctatagcaga tcaggctgac taagagaaac 180
cccataagag ttacatactc attaatctcc gtctctatcc ccaggtctca gatgctggac 240
aaggtgtca 249

<210> 149
<211> 255

<212> DNA
<213> Homo sapien

<400> 149
tgacaccttg tccagcatct gctattttgt gacttttaa taatagccat tctgactggt 60
gtgagatggt aactcattgt gggtttggtc tgcatttctc taatgatcag tgatattaag 120
ctttttttaa atatgcttgt tgaccacatg tatatcatct tttgagaagt gtctgttcat 180
atcctttgcc cacttttaa ttttttatc ttgtaaattt gtttaatttc cttacagatg 240
ctggacaagg tgtca 255

<210> 150
<211> 318
<212> DNA
<213> Homo sapien

<400> 150
ttacgctgca acactgtgga ggccaagctg ggatcacttc ttcattctaa ctggagagga 60
gggaagttca agtccagcag agggtgggtg ggtagacagt ggcactcaga aatgtcagct 120
ggacccctgt ccccgcatag gcaggacagc aaggctgtgg ctctccaggg ccagctgaag 180
aacaggacac tgtctccgct gccacaaagc gtcagagact cccatctttg aagcacggcc 240
ttcttggtct tcctgcactt ccctgttctg ttagagacct ggttatagac aaggcttctc 300
cacagtgttg cagcgtaa 318

<210> 151
<211> 323
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(323)
<223> n = A,T,C or G

<400> 151tnacgcngcn acnntgtaga ganggnaagg cnttccccac attnccccctt
catnanagaa 60
ttattcnacc aagnntgacc natgccnttt atgacttaca tgcnnactnc ntaatctgtn 120
tcnngcctta aaagcnnntc cactacatgc ntcancactg tntgtgtnac ntcatnaact 180
gtcngnaata ggggcncata actacagaaa tgcanttcat actgcttcca ntgccatcng 240
cgtgtggcct tncctactct tcttntattc caagtagcat ctctggantg cttccccact 300
ctccacattg ttgcagcnat aat 323

<210> 152
<211> 311
<212> DNA
<213> Homo sapien

<400> 152
tcaagattcc ataggctgac cagtccaagg agagttgaaa tcatgaagga gagtctatct 60
ggagagagct gtagttttga gggttgcaaa gacttaggat ggagttggtg ggtgtggtta 120
gtctctaagg ttgattttgt tcataaattt catgccctga atgccttgct tgcctcaccc 180
tggtccaagc cttagtgaac acctaaaagt ctctgtcttc ttgctctcca aacttctcct 240

gaggatttcc tcagattgtc tacattcaga tcgaagccag ttggcaaaca agatgcagtc 300
cagagggtca g 311

<210> 153
<211> 332
<212> DNA
<213> Homo sapien

<400> 153
caagattcca taggctgacc aggaggctat tcaagatctc tggcagttga ggaagtctct 60
ttaagaaaat agtttaaaca atttgttaaa atttttctgt cttacttcat ttctgtagca 120
gttgatatct ggctgtcctt tttataatgc agagtgggaa ctttccctac catgtttgat 180
aaatgttgtc caggctccat tgccaataat gtgttgtcca aaatgcctgt ttagttttta 240
aagacggaac tccacccttt gcttggtctt aagtatgtat ggaatgttat gataggacat 300
agtagtagcg gtggtcagcc tatggaatct tg 332

<210> 154
<211> 345
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(345)
<223> n = A,T,C or G

<400> 154
tcaagattcc ataggctgac ctggacagag atctcctggg tctggcccag gacagcaggc 60
tcaagctcag tggagaaggt ttccatgacc ctcagattcc cccaaacctt ggattgggtg 120
acattgcatc tcctcagaga gggaggagat gtangtctgg gcttccacag ggacctggta 180
ttttaggatc agggtaccgc tggcctgagg cttggatcat tcanagcctg ggggtggaat 240
ggctggcagc ctgtggcccc attgaaatag gctctggggc actccctctg ttcctanttg 300
aacttgggta aggaacagga atgtggtcan cctatggaat cttga 345

<210> 155
<211> 295
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(295)
<223> n = A,T,C or G

<400> 155
gacgcttggc cacttgacac attaaacagt tttgcataat cactancatg tatttctagt 60
ttgctgtctg ctgtgatgcc ctgccctgat tctctggcgt taatgatggc aagcataatc 120
aaacgctgtt ctgttaattc caagttataa ctggcattga ttaaagcatt atctttcaca 180
actaaactgt tcttcatana acagcccata ttattatcaa attaagagac aatgtattcc 240
aatatccttt anggccaata tatttnatgt cccttaatta agagctactg tccgt 295

<210> 156
<211> 406
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(406)
<223> n = A,T,C or G

<400> 156
gacgcttggc cacttgacac tgcagtggga aaaccagcat gagccgctgc ccccaaggaa 60
cctcgaagcc caggcagagg accagccatc ccagcctgca ggtaaagtgt gtcacctgtc 120
aggtgggctt ggggtgagtg ggtgggggaa gtgtgtgtgc aaaggggtg tnaatgtnta 180
tgcgtgtgag catgagtgat ggctagtgtg actgcatgtc agggagtgtg aacaagcgtg 240
cgggggtgtg tgtgcaagtg cgtatgcata tgagaatatg tgtctgtgga tgagtgcatt 300
tgaaagtctg tgtgtgtgcg tgtggtcatg anggtaantt antgactgcg caggatgtgt 360
gagtgtgcat ggaacactca ntgtgtgtgt caagtggccn ancgtc 406

<210> 157
<211> 208
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(208)
<223> n = A,T,C or G

<400> 157
tgacgcttgg ccacttgaca cactaaaggg tgttactcat cactttcttc tctcctcggt 60
ggcatgtgag tgcatctatt cacttggcac tcatttgttt ggcagtgact gtaanccana 120
tctgatgcat acaccagctt gtaaattgaa taaatgtctc taatactatg tgctcacaat 180
anggtanggg tgaggagaag gggagaga 208

<210> 158
<211> 547
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(547)
<223> n = A,T,C or G

<400> 158
cttcaacctc cttcaacctc cttcaacctc ctggattcaa acaatcatcc cacctcagac 60
tccttagtag ctgagactac agactcacgc cactacatct ggctaaattt ttgtagagat 120
agggtttcat catgttgccc tggctggtct caaactcctg acctcaagca atgtgcccac 180
ctcagcctcc caaagtgctg ggattacagg cataagccac catgcccagt ccatntttaa 240
tctttcctac cacattctta ccacactttc ttttatgttt agatacataa atgcttacca 300

ttatgataca attgcccaca gtattaagac agtaacatgc tgcacaggtt tgtagcctag 360
gaacagtagg caataccaca tagcttaggt gtgtggtaga ctataccatc taggtttgtg 420
taagttacac tttatgctgt ttacacaatg acaaaaccat ctaatgatgc atttctcaga 480
atgtatcctt gtcagtaagc tatgatgtac agggaacact gcccaaggac acagatattg 540
tacctgt 547

<210> 159
<211> 203
<212> DNA
<213> Homo sapien

<400> 159
gctcctcttg ccttaccaac tcacccagta tgtcagcaat tttatcrgct ttacctacga 60
aacagcctgt atccaaacac ttaacacact cacctgaaaa gttcaggcaa caatcgcctt 120
ctcatgggtc tctctgctcc agttctgaac ctttctcttt tcctagaaca tgcatttarg 180
tcgatagaag ttcctctcag tgc 203

<210> 160
<211> 402
<212> DNA
<213> Homo sapien

<400> 160
tgtaagtcga gcagtgtgat gggtggaaca gggttgtaag cagtaattgc aaactgtatt 60
taaacaataa taataatatt tagcatttat agagcacttt atatcttcaa agtacttgca 120
aacattayct aattaaatac cctctctgat tataatctgg atacaaatgc acttaaactc 180
aggacagggt catgagaraa gtatgcattt gaaagttggt gctagctatg ctttaaaaac 240
ctatacaatg atgggraagt tagagttcag attctgttgg actgtttttg tgcatttcag 300
ttcagcctga tggcagaatt agatcatatc tgcactcgat gactytgctt gataacttat 360
cactgaaatc tgagtgttga tcatcacact gctcgactta ca 402

<210> 161
<211> 193
<212> DNA
<213> Homo sapien

<400> 161
agcatgttga gcccagacac tgaccaggag aaaaaccaac caatagaaac acgcccagac 60
actgaccagg agaaaaacca accaataaaa acaggcccgg acataagaca aataataaaa 120
ttagcggaca aggacatgaa aacagctatt gtaagagcgg atatagtggt gtgtgtctgg 180
gctcaacatg cta 193

<210> 162
<211> 147
<212> DNA
<213> Homo sapien

<400> 162
tgttgagccc agacactgac caggagaaaa accaaccaat aaaaacaggc ccggacataa 60
gacaaataat aaaattagcg gacaaggaca tgaaaacagc tattgtaaga gcggatatag 120
tggtgtgtgt ctgggctcaa catgcta 147

<210> 163
<211> 294
<212> DNA
<213> Homo sapien

<400> 163
tagcatgttg agcccagaca caaatctttc cttaagcaat aaatcatttc tgcatatgtt 60
tttaaaacca cagctaagcc atgattattc aaaaggacta ttgtattggg tattttgatt 120
tgggttctta tctccctcac attatcttca tttctatcat tgacctctta tcccagagac 180
tctcaaactt ttatgttata caaatcacat tctgtctcaa aaaatatctc acccacttct 240
cttctgtttc tgcgtgtgta tgtgtgtgtg tgtgtgtctg ggctcaacat gcta 294

<210> 164
<211> 412
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(412)
<223> n = A,T,C or G

<400> 164
cgggattggc tttgagctgc agatgctgcc tgtgaccgca cccggcgtgg aacagaaagc 60
cacctggctg caagtgcgcc agagccgccc tgactacgtg ctgctgtggg gctggggcgt 120
gatgaactcc accgccctga aggaagccca ggccaccgga tacccccgcg acaagatgta 180
cggcgtgtgg tgggccggtg cggagcccga tgtgcgtgac gtgggcgaag gcgccaaggg 240
ctacaacgcg ctggctctga acggctacgg cacgcagtcc aaggtgatcc angacatcct 300
gaaacacgtg cacgacaagg gccagggcac ggggcccaaa gacgaagtgg gctcggtgct 360
gtacacccgc ggcgtgatca tccagatgct ggacaaggtg tcaatcacta at 412

<210> 165
<211> 361
<212> DNA
<213> Homo sapien

<400> 165
ttgacacctt gtccagcatc tgcatctgat gagagcctca gatggctacc actaatggca 60
gaaggcaaag gagaacaggc attgtatggc aagaaaggaa gaaagagaga ggggagaaag 120
gtgctaggtt cttttcaaca accagttctt gatggaactg agagtaagag ctcaaggcca 180
ggtgtggtga ctccaaccag taatcccaac attttaggag gctgaggcag gcagatgtct 240
tgaccccatg agtttgtgac cagcctgaac aacatcatga gactccatct ctacaataat 300
tacaaaaatt aatcaggcat tgtggtatgc cctgtagtcc cagatgctgg acaaggtgtc 360
a 361

<210> 166
<211> 427
<212> DNA
<213> Homo sapien

<400> 166
twgactgact catgtcccct acacccaact atcttctcca ggtggccagg catgatagaa 60
tctgatcctg acttagggga tatttttctt tttacttccc atcttgattc cctgccggtg 120
agtttcctgg ttcagggtaa gaaaggagct caggccaaag taatgaacaa atccatcctc 180
acagacgtac agaataagag aacwtggacw tagccagcag aacmcaaktg aaamcagaac 240
mcttamctag gatracaamc mcrraratar ktgcycmcmc wtataataga aaccaaactt 300
gtatctaatt aaatatttat ccacygtcag ggcattagtg gttttgataa atacgctttg 360
gctaggattc ctgaggttag aatggaaraa caattgcamc gagggtaggg gacatgagtc 420
aktctaa 427

<210> 167
<211> 500
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(500)
<223> n = A,T,C or G

<400> 167
aacgtcgcat gctcccggcc gccatggccg cgggatagac tgactcatgt cccctaagat 60
agaggagaca cctgctaggt gtaaggagaa gatggttagg tctacggagg ctccagggtg 120
ggagtagttc cctgctaagg gagggtagac tgttcaacct gttcctgctc cggcctccac 180
tatagcagat gcgagcagga gtaggagaga gggaggtaag agtcagaagc ttatgttgtt 240
tatgcgggga aacgccrtat cgggggcagc cragttatta ggggacantr tagwyartcw 300
agntagcatc caaagcgngg gagttntccc atatggttgg acctgcaggc ggccgcatta 360
gtgattagca tgtgagcccc agacacgcat agcaacaagg acctaaactc agatcctgtg 420
ctgattactt aacatgaatt attgtattta tttaacaact ttgagttatg aggcatatta 480
ttaggtccat attacctgga 500

<210> 168
<211> 358
<212> DNA
<213> Homo sapien

<400> 168
ttcatcgctc ggtgactcaa gcctgtaatc ccagaacttt gggaggccga ggggagcaga 60
tcacctgagg ttgggagttt gagaccagcc tggccaacat ggtgacaacc cgtctctgct 120
aaaaatacaa aaattagcca agcatggtgg catgcacttg taatcccagc tactcgggag 180
gctgaggcag gagaatcact tgaggccagg aggcagaggt tgcagtgagg cagaggttga 240
gatcatgcca ctgcactcca gcctgggcaa cagagtaaga ctccatctca aaaaaaaaaa 300
aaaaaaagaa tgatcagagc cacaaataca gaaaaccttg agtcaccgag cgatgaaa 358

<210> 169
<211> 1265
<212> DNA
<213> Homo sapien

<400> 169
ttctgtccac accaatctta gagctctgaa agaatttgtc tttaaatatc ttttaatagt 60

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aacatgtatt ttatggacca aattgacatt ttcgactatt ttttcccaaa aaaagtcagg    120
tgaatttcag cacactgagt tgggaatttc ttatcccaga agwcggcacg agcaatttca    180
tatttattta agattgattc catactccgt tttcaaggag aatccctgca gtctccttaa    240
aggtagaaca aatactttct attttttttt caccattgtg ggattggact ttaagaggtg    300
actctaaaaa aacagagaac aaatatgtct cagttgtatt aagcacggac ccatattatc    360
atattcactt aaaaaaatga tttcctgtgc accttttggc aacttctctt ttcaatgtag    420
ggaaaaactt agtcaccctg aaaacccaca aaataaataa aacttgtaga tgtgggcaga    480
argtttgggg gtggacattg tatgtgttta aattaaaccc tgtatcactg agaagctgtt    540
gtatgggtca gagaaaatga atgcttagaa gctgttcaca tcttcaagag cagaagcaaa    600
ccacatgtct cagctatatt attatttatt ttttatgcat aaagtgaatc atttcttctg    660
tattaatttc caaagggttt taccctctat ttaaatgctt tgaaaaacag tgcattgaca    720
atgggttgat atttttcttt aaaagaaaaa tataattatg aaagccaaga taatctgaag    780
cctgttttat tttaaaactt tttatgttct gtggttgatg ttgtttgttt gtttgtttct    840
attttgttgg ttttttactt tgtttttgt  tttgttttgt tttggtttdg catactacat    900
gcagtttctt taaccaatgt ctgtttggct aatgtaatta aagttgttaa tttatatgag    960
tgcatttcaa ctatgtcaat ggtttcttaa tatttattgt gtagaagtac tggtaatttt   1020
tttatttaca atatgtttaa agagataaca gtttgatatg ttttcatgtg tttatagcag   1080
aagttattta tttctatggc attccagcgg atattttggt gtttgcgagg catgcagtca   1140
atattttgta cagttagtgg acagtattca gcaacgcctg atagcttctt tggccttatg   1200
ttaaataaaa agacctgttt gggatgtaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa   1260
aaaaa                                                               1265
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<210> 170
<211> 383
<212> DNA
<213> Homo sapien

<400> 170

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tgtaagtcga gcagtgtgat gacgatattc ttcttattaa tgtggtaatt gaacaaatga     60
tctgtgatac tgatcctgag ctaggaggcg ctgttcagtt aatgggactt cttcgtactc    120
taattgatcc agagaacatg ctggctacaa ctaataaaac cgaaaaaagt gaatttctaa    180
atttttcta  caaccattgt atgcatgttc tcacagcacc acttttgacc aatacttcag    240
aagacaaatg tgaaaaggat aatatagttg gatcaaacaa aaacaacaca atttgtcccg    300
ataattatca aacagcacag ctacttgcct taattttaga gttactcaca ttttgtgtgg    360
aacatcacac tgctcgactt aca                                            383
```

<210> 171
<211> 383
<212> DNA
<213> Homo sapien

<400> 171

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tgggcacctt caatatcgca agttaaaaat aatgttgagt ttattatact tttgacctgt     60
ttagctcaac agggtgaagg catgtaaaga atgtggactt ctgaggaatt ttcttttaaa    120
aagaacataa tgaagtaaca ttttaattac tcaaggacta cttttggttg aagtttataa    180
tctagatacc tctacttttt gtttttgctg ttcgacagtt cacaaagacc ttcagcaatt    240
tacagggtaa aatcgttgaa gtagtggagg tgaaactgaa atttaaaatt attctgtaaa    300
tactataggg aaagaggctg agcttagaat cttttggttg ttcatgtgtt ctgtgctctt    360
atcatcacac tgctcgactt aca                                            383
```

<210> 172

<211> 699
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(699)
<223> n = A,T,C or G

<400> 172
tcgggtgatg cctcctcagg cttgtcgtta gtgtacacag agctgctcat gaagcgacag 60
cggctgcccc tggcacttca gaacctcttc ctctacactt ttggtgcgct tctgaatcta 120
ggtctgcatg ctggcggcgg ctctggccca ggcctcctgg aaagtttctc aggatgggca 180
gcactcgtgg tgctgagcca ggcactaaat ggactgctca tgtctgctgt catggagcat 240
ggcagcagca tcacacgcct ctttgtggtg tcctgctcgc tggtggtcaa cgccgtgctc 300
tcagcagtcc tgctacggct gcagctcaca gccgccttct tcctggccac attgctcatt 360
ggcctggcca tgcgcctgta ctatggcagc cgctagtccc tgacaacttc caccctgatt 420
ccggaccctg tagattgggc gccaccacca gatccccctc ccaggccttc ctccctctcc 480
catcagcggc cctgtaacaa gtgccttgtg agaaaagctg gagaagtgag ggcagccagg 540
ttattctctg gaggttggtg gatgaagggg taccctagg agatgtgaag tgtgggtttg 600
gttaaggaaa tgcttaccat cccccacccc caaccaagtt nttccagact aaagaattaa 660
ggtaacatca atacctaggc ctgaggaggc atcacccga 699

<210> 173
<211> 701
<212> DNA
<213> Homo sapien

<400> 173
tcgggtgatg cctcctcagg ccagatcaaa cttggggttg aaaactgtgc aaagaaatca 60
atgtcggaga aagaattttg caaaagaaaa atgcctaatc agtactaatt taataggtca 120
cattagcagt ggaagaagaa atgttgatat tttatgtcag ctattttata atcaccagag 180
tgcttagctt catgtaagcc atctcgtatt cattagaaat aagaacaatt ttattcgtcg 240
gaaagaactt ttcaatttat agcatcttaa ttgctcagga ttttaaattt tgataaagaa 300
agctccactt ttggcaggag tagggggcag ggagagagga ggctccatcc acaaggacag 360
agacaccagg gccagtaggg tagctggtgg ctggatcagt cacaacggac tgacttatgc 420
catgagaaga aacaacctcc aaatctcagt tgcttaatac aacacaagct catttcttgc 480
tcacgttaca tgtcctatgt agatcaacag caggtgactc agggacccag gctccatctc 540
catatgagct tccatagtca ccaggacacg ggctctgaaa gtgtcctcca tgcagggaca 600
catgcctctt cctttcattg ggcagagcaa gtcacttatg gccagaagtc acactgcagg 660
gcagtgccat cctgctgtat gcctgaggag gcatcacccg a 701

<210> 174
<211> 700
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(700)
<223> n = A,T,C or G

<400> 174
tcgggtgatg cctcctcang cccctaaatc agagtccagg gtcagagcca caggagacag 60
ggaaagacat agattttaac cggccccctt caggagattc tgaggctcag ttcactttgt 120
tgcagtttga acagaggcag caaggctagt ggttaggggc acggtctcta aagctgcact 180
gcctggatct gcctcccagc tctgccagga accagctgcg tggccttgag ctgctgacac 240
gcagaaagcc ccctgtggac ccagtctcct cgtctgtaag atgaggacag gactctagga 300
acccttccc ttggtttggc ctcactttca caggctccca tcttgaactc tatctactct 360
tttcctgaaa ccttgtaaaa gaaaaaagtg ctagcctggg caacatggca aaaccctgtc 420
tctacaaaaa atacaaaaat tagttgggtg tggtggcatg tgcctgtagt cccagccact 480
tgggaggtgc tgaggtggga ggatcacttg agcccgggag gtggaggttg cagtgagcca 540
agatcatgcc actgcactcc agcctgagta atagagtaag actctgtctc aaaaacaaca 600
acaacaacag tgagtgtgcc tctgtttccg ggttggatgg ggcaccacat ttatgcatct 660
ctcagatttg gacgctgcag cctgaggagg catcacccga 700

<210> 175
<211> 484
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(484)
<223> n = A,T,C or G

<400> 175
tatagggcga attgggcccg agttgcatgn tcccggccgc catggccgcg ggattcgggt 60
gatgcctcct caggcttgtc tgccacaagc tacttctctg agctcagaaa gtgccccttg 120
atgagggaaa atgtcctact gcactgcgaa tttctcagtt ccattttacc tcccagtcct 180
ccttctaaac cagttaataa attcattcca caagtattta ctgattacct gcttgtgcca 240
gggactattc tcaggctgaa gaaggtggga ggggagggcg gaacctgagg agccacctga 300
gccagcttta tatttcaacc atggctggcc catctgagag catctcccca ctctcgccaa 360
cctatcgggg catagcccag ggatgccccc aggcggccca ggttagatgc gtccctttgg 420
cttgtcagtg atgacataca ccttagctgc ttagctggtg ctggcctgag gaggcatcac 480
ccga 484

<210> 176
<211> 432
<212> DNA
<213> Homo sapien

<400> 176
tcgggtgatg cctcctcagg gctcaaggga tgagaagtga cttctttctg gagggaccgt 60
tcatgccacc caggatgaaa atggataggg acccacttgg aggacttgct gatatgtttg 120
gacaaatgcc aggtagcgga attggtactg gtccaggagt tatccaggat agattttcac 180
ccaccatggg acgtcatcgt tcaaatcaac tcttcaatgg ccatgggggа cacatcatgc 240
ctcccacaca atcgcagttt ggagagatgg gaggcaagtt tatgaaaagc caggggctaa 300
gccagctcta ccataaccag agtcagggac tcttatccca gctgcaagga cagtcgaagg 360
atatgccacc tcggttttct aagaaaggac agcttaatgc agatgagatt agcctgagga 420
ggcatcaccc ga 432

<210> 177
<211> 788
<212> DNA
<213> Homo sapien

<400> 177
tagcatgttg agcccagaca cagtagcatt tgtgccaatt tctggttgga atggtgacaa 60
catgctggag ccaagtgcta acatgccttg gttcaaggga tggaaagtca cccgtaagga 120
tggcaatgcc agtggaacca cgctgcttga ggctctggac tgcatcctac caccaactcg 180
cccaactgac aagcccttgc gcctgcctct ccaggatgtc tacaaaattg gtggtattgg 240
tactgttcct gttggccgag tggagactgg tgttctcaaa cccggtatgg tggtcacctt 300
tgctccagtc aacgttacaa cggaagtaaa atctgtcgaa atgcaccatg aagctttgag 360
tgaagctctt cctggggaca atgtgggctt caatgtcaag aatgtgtctg tcaaggatgt 420
tcgtcgtggc aacgttgctg gtgacagcaa aaatgaccca ccaatggaag cagctggctt 480
cactgctcag gtgattatcc tgaaccatcc aggccaaata agtgccggct atgcccctgt 540
attggattgc cacacggctc acattgcatg caagtttgct gagctgaagg aaaagattga 600
tcgccgttct ggtaaaaagc tggaagatgg ccctaaattc ttgaagtctg gtgatgctgc 660
cattgttgat atggttcctg gcaagcccat gtgtgttgag agcttctcag actatccacc 720
tttgggtcgc tttgctgttc gtgatatgag acagacagtt gcggtgggtg tctgggctca 780
acatgcta 788

<210> 178
<211> 786
<212> DNA
<213> Homo sapien

<400> 178
tagcatgttg agcccagaca cctgtgtttc tgggagctct ggcagtggcg gattcatagg 60
cacttgggct gcactttgaa tgacacactt ggctttatta gattcactag tttttaaaaa 120
attgttgttc gtttcttttc attaaaggtt taatcagaca gatcagacag cataattttg 180
tatttaatga cagaaacgtt ggtacatttc ttcatgaatg agcttgcatt ctgaagcaag 240
agcctacaaa aggcacttgt tataaatgaa agttctggct ctagaggcca gtactctgga 300
gtttcagagc agccagtgat tgttccagtc agtgatgcct agttatatag aggaggagta 360
cactgtgcac tcttctaggt gtaagggtat gcaactttgg atcttaaaat tctgtacaca 420
tacacacttt atatatatgt atgtatgtat gaaaacatga aattagtttg tcaaatatgt 480
gtgtgtttag tattttagct tagtgcaact atttccacat tatttattaa attgatctaa 540
gacactttct tgttgacacc ttgaatatta atgttcaagg gtgcaatgtg tattccttta 600
gattgttaaa gcttaattac tatgatttgt agtaaattaa cttttaaaat gtatttgagc 660
ccttctgtag tgtcgtaggg ctcttacagg gtgggaaaga ttttaatttt ccagttgcta 720
attgaacagt atggcctcat tatatatttt gatttatagg agtttgtgtc tgggctcaac 780
atgcta 786

<210> 179
<211> 796
<212> DNA
<213> Homo sapien

<400> 179
tagcatgttg agcccagaca ctggttacaa gaccagacct gcttcctcca tatgtaaaca 60
gcttttaaaa agccagtgaa ccttttttaat actttggcaa ccttctttca caggcaaaga 120
acacccccat ccgccccttg tttggagtgc agagtttggc tttggttctt tgccttgcct 180

ggagtatact tctaattcct gttgtcctgc acaagctgaa taccgagcta cccaccgcca 240
cccaggccag gtttccactc atttattact ttatgtttct gttccattgc tggtccacag 300
aaataagttt tcctttggag gaatgtgatt ataccccttt aatttcctcc ttttgctttt 360
ttttaatatc attggtatgt gtttggccca gaggaaactg aaattcacca tcatcttgac 420
tggcaatccc attaccatgc tttttttaaa aaacgtaatt tttcttgcct tacattggca 480
gagtagccct tcctggctac tggcttaatg tagtcactca gtttctaggt ggcattaggc 540
atgagacctg aagcacagac tgtcttacca caaaaggtga caagatctca aaccttagcc 600
aaagggctat gtcaggtttc aatgctatct gcttctgttc ctgctcactg ttctggattt 660
tgtccttctt catccctagc accagaattt cccagtctcc ctccctacct tcccttgttt 720
taattctaat ctatcagcaa aataactttt caaatgtttt aaccggtatc tccatgtgtc 780
tgggctcaac atgcta 796

<210> 180
<211> 488
<212> DNA
<213> Homo sapien

<400> 180
ggatgtgctg caaggcgatt aagttgggta acgccagggt tttcccagtc acgacgttgt 60
aaaacgacgg ccagtgaatt gtaatacgac tcactatagg gcgaattggg cccgacgtcg 120
catgctcccg gccgccatgg ccgcgggata gcatgttgag cccagacacc tgcaggtcat 180
ttggagagat ttttcacgtt accagcttga tggtcttttt caggaggaga gacactgagc 240
actcccaagg tgaggttgaa gatttcctct agatagccgg ataagaagac taggagggat 300
gcctagaaaa tgattagcat gcaaatttct acctgccatt tcagaactgt gtgtcagccc 360
acattcagct gcttcttgtg aactgaaaag agagaggtat tgagactttt ctgatggccg 420
ctctaacatt gtaacacagt aatctgtgtg tgtgtgggtg tgtgtgtgtg tctgggctca 480
acatgcta 488

<210> 181
<211> 317
<212> DNA
<213> Homo sapien

<400> 181
tagcatgttg agcccagaca cggcgacggt acctgatgag tggggtgatg gcacctgtga 60
aaaggaggaa cgtcatcccc catgatattg gggacccaga tgatgaacca tggctccgcg 120
tcaatgcata tttaatccat gatactgctg attggaagga cctgaacctg aagtttgtgc 180
tgcaggttta tcgggactat tacctcacgg gtgatcaaaa cttcctgaag gacatgtggc 240
ctgtgtgtct agtaagggat gcacatgcag tggccagtgt gccaggggta tggttggtgt 300
ctgggctcaa catgcta 317

<210> 182
<211> 507
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(507)
<223> n = A,T,C or G

<400> 182
tagcatgttg agcccagaca ctggctgtta gccaaatcct ctctcagctg ctccctgtgg 60
tttggtgact caggattaca gaggcatcct gtttcaggga acaaaaagat tttagctgcc 120
agcagagagc accacataca ttagaatggt aaggactgcc acctccttca agaacaggag 180
tgagggtggt ggtgaatggg aatggaagcc tgcattccct gatgcatttg tgctctctca 240
aatcctgtct tagtcttagg aaaggaagta aagtttcaag gacggttccg aactgctttt 300
tgtgtctggg ctcaacatgc tatcccgcgg ccatggcggc cgggagcatg cgacgtcggg 360
cccaattcgc cctatagtga gtcgtattac aattcactgg ccgtcgtttt acaacgtcgt 420
gactgggaaa accctggcgt tacccaactt aatcgccttg cagcacatcc cctttccca 480
gctggcgtaa tancgaaaag gcccgca 507

<210> 183
<211> 227
<212> DNA
<213> Homo sapien

<400> 183
gatttacgct gcaacactgt ggaggtagcc ctggagcaag gcaggcatgg atgcttctgc 60
aatccccaaa tggagcctgg tatttcagcc aggaatctga gcagagcccc ctctaattgt 120
agcaatgata agttattctc tttgttcttc aaccttccaa tagccttgag cttccagggg 180
agtgtcgtta atcattacag cctggtctcc acagtgttgc agcgtaa 227

<210> 184
<211> 225
<212> DNA
<213> Homo sapien

<400> 184
ttacgctgca acactgtgga gcagattaac atcagacttt tctatcaaca tgactggggt 60
tactaaaaag acaacaaatc aatggcttca aaagtctaag gaataatttc gatacttcaa 120
ctttataaaa cctgacaaaa ctatcaatca agcataaaga cagatgaaga acatttccag 180
attttggcca atcagatatt ttacctccac agtgttgcag cgtaa 225

<210> 185
<211> 597
<212> DNA
<213> Homo sapien

<400> 185
ggcccgacgt cgcatgctcc cggccgccat ggccgcggga ttcgttaggg tctctatcca 60
ctgggaccca taggctagtc agagtattta gagttgagtt cctttctgct tcccagaatt 120
tgaaagaaaa ggagtgaggt gatagagctg agagatcaga tttgcctctg aagcctgttc 180
aagatgtatg tgctcagacc ccaccactgg ggcctgtggg tgaggtcctg ggcatctatt 240
tgaatgaatt gctgaagggg agcactatgc caaggaaggg gaacccatcc tggcactggc 300
acaggggtca ccttatccag tgctcagtgc ttctttgctg ctacctggtt ttctctcata 360
tgtgaggggc aggtaagaag aagtgcccrg tgttgtgcga gttttagaac atctaccagt 420
aagtggggaa gtttcacaaa gcagcagctt tgttttgtgt attttcacct tcagttagaa 480
gaggaaggct gtgagatgaa tgttagttga gtggaaaaga cgggtaagct tagtggatag 540
agaccctaac gaatcactag tgcggccgcc ttgcaggtcg accatatggg agagctc 597

<210> 186

<211> 597
<212> DNA
<213> Homo sapien

<400> 186
ggcccgaagt tgcatgttcc cggccgccat ggccgcggga ttcgttaggg tctctatcca 60
ctacctaaaa aatcccaaac atataactga actcctcaca cccaattgga ccaatccatc 120
accccagagg cctacagatc ctcctttgat acataagaaa atttccccaa actacctaac 180
tatatcattt tgcaagattt gttttaccaa attttgatgg cctttctgag cttgtcagtg 240
tgaaccacta ttacgaacga tcggatatta actgcccctc accgtccagg tgtagctggc 300
aacatcaagt gcagtaaata ttcattaagt tttcacctac taaggtgctt aaacacccta 360
gggtgccatg tcggtagcag atcttttgat ttgtttttat ttcccataag ggtcctgttc 420
aaggtcaatc atacatgtag tgtgagcagc tagtcactat cgcatgactt ggagggtgat 480
aatagaggcc tcctttgctg ttaaagaact cttgtcccag cctgtcaaag tggatagaga 540
ccctaacgaa tcactagtgc ggccgcctgc aggtcgacca tatgggagag ctcccaa 597

<210> 187
<211> 324
<212> DNA
<213> Homo sapien

<400> 187
tcgttagggt ctctatccac ttgcaggtaa aatccaatcc tgtgtatatc ttatagtctt 60
ccatatgtag tggttcaaga gactgcagtt ccagaaagac tagccgagcc catccatgtc 120
ttccacttaa ccctgctttg ggttacacat cttaactttt ctgttcaagt ttctctgtgt 180
agtttatagc atgagtattg ggawaatgcc ctgaaacctg acatgagatc tgggaaacac 240
aaacttactc aataagaatt tctcccatat ttttatgatg gaaaaatttc acatgcacag 300
aggagtggat agagacccta acga 324

<210> 188
<211> 178
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(178)
<223> n = A,T,C or G

<400> 188
gcgcggggat tcggggtgat acctcctcat gccaaaatac aacgtntaat ttcacaactt 60
gccttccaat ttacgcattt tcaatttgct ctccccattt gttgagtcac aacaaacacc 120
attgcccaga aacatgtatt acctaacatg cacatactct taaaactact catccctt 178

<210> 189
<211> 367
<212> DNA
<213> Homo sapien

<400> 189
tgacaccttg tccagcatct gacacagtct tggctcttgg aaaatattgg ataaatgaaa 60

atgaatttct ttagcaagtg gtataagctg agaatatacg tatcacatat cctcattcta 120
agacacattc agtgtccctg aaattagaat aggacttaca ataagtgtgt tcactttctc 180
aatagctgtt attcaattga tggtaggcct taaaagtcaa agaaatgaga gggcatgtga 240
aaaaaagctc aacatcactg atcattagaa aacttccatt caaaccccca atgagatacc 300
atctcatacc agtcagaatg gctattatta aaaagtcaaa aaataacaga tgctggacaa 360
ggtgtca 367

<210> 190
<211> 369
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(369)
<223> n = A,T,C or G

<400> 190
gacaccttgt ccagcatctg acaacgctaa cagcctgagg agatctttat ttatttattt 60
agtttttact ctggctaggc agatggtggc taaaacattc atttacccat ttattcattt 120
aattgttcct gcaaggccta tggatagagt attgtccagc actgctctgg aagctaggag 180
catggggatg aacaagatag gctacatcct gttcccacag aacttccact ttagtctggg 240
aaacagatga tatatacaaa tatataaatg aattcaggta gttttaagta cgaaaagaat 300
aagaaagcag agtcatgatt tanaatgctg gaaacagggg ctattgcttg agatattgaa 360
ggtgcccaa 369

<210> 191
<211> 369
<212> DNA
<213> Homo sapien

<400> 191
tgacaccttg tccagcatct gcacagggaa aagaaactat tatcagagtg aacaggcaac 60
ctacagaatg ggagaaaatt tttgcaatct atccatctga caaagggcta atatccagaa 120
tctacaaaga acttatacaa atttacaaga aacaaacaaa caaacaactc ctcaaaaagt 180
gggtgaagga tgtgaacaga cacttctcaa aagaagacat ttatggggcc aacaaacata 240
tgaaaaaaag ctcatcatca ctggtcacta gataaatgca aatcaaaacc acaatgagat 300
accatctcat tccagttaga atggcaatca ttaaaaagtc aggaaacaac agatgctgga 360
caaggtgtc 369

<210> 192
<211> 449
<212> DNA
<213> Homo sapien

<400> 192
tgacgcttgg ccacttgaca cttcatcttt gcacagaaaa acttctttac agatttaatt 60
caagactggt ctagtgacag tcctccagac atttttttcat ttgttccata tacgtggaat 120
tttaaaatca tgtttcatca gtttgaaatg atttgggctg ctaatcaaca caattggatc 180
gactgttcta ctaaacaaca ggaaaatgtg tatctggcag cctgtggaga aacactaaac 240
attgattttt ctttgccttt tacggacttt gttccagcta catgtaatac caagttctct 300

ttaagaggag aagatgttga tcttcatttg tttctaccag actgccaccc tagtaaatat 360
tctttattta tgctggtaaa aaattgccat ccaaataaga tgattcatga tactggtatt 420
cctgctgagt gtcaagtggc caagcgtca 449

<210> 193
<211> 372
<212> DNA
<213> Homo sapien

<400> 193
tgacgcttgg ccacttgaca ccagggatgt akcagttgaa tataatcctg caattgtaca 60
tattggcaat ttcccatcaa acattctaga aagagacaac caggattgct aggccataaa 120
agctgcaata aataactggt aattgcagta atcatttcag gccaattcaa tccagtttgg 180
ctcagaggtg cctttggctg agagaagagg tgagatataa tgtgttttct tgcaacttct 240
tggaagaata actccacaat agtctgagga ctagatacaa acctatttgc cattaaagca 300
ccagagtctg ttaattccag tactgataag tgttggagat tagactccag tgtgtcaagt 360
ggccaagcgt ca 372

<210> 194
<211> 309
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(309)
<223> n = A,T,C or G

<400> 194
tgacgcttgg ccacttgaca cttatgtaga atccatcgtg ggctgatgca agccctttat 60
ttaggcttag tgttgtgggc accttcaata tcacactaga gacaaacgcc acaagatctg 120
cagaaacatt cagttctgan cactcgaatg gcaggataac tttttgtgtt gtaatccttc 180
acatatacaa aaacaaactc tgcantctca cgttacaaaa aaacgtactg ctgtaaaata 240
ttaagaaggg gtaaaggata ccatctataa caaagtaact tacaactagt gtcaagtggc 300
caagcgtca 309

<210> 195
<211> 312
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(312)
<223> n = A,T,C or G

<400> 195
tgacgcttgg ccacttgaca cccaatctcg cacttcatcc tcccagcacc tgatgaagta 60
ggactgcaac tatccccact tcccagatga ggggaccaan gtacacatta ggacccggat 120
gggagcacag atttgtccga tcccagactc caagcactca gcgtcactcc aggacagcgg 180
ctttcagata aggtcacaaa catgaatggc tccgacaacc ggagtcagtc cgtgctgagt 240

taaggcaatg gtgacacgga tgcacgtgtn acctgtaatg gttcatcgta agtgtcaagt 300
ggccaagcgt ca 312

<210> 196
<211> 288
<212> DNA
<213> Homo sapien

<400> 196
tgtatcgacg tagtggtctc ctcagccatg cagaactgtg actcaattaa acctctttcc 60
tttatgaatt acccaatctc gggtagtgtc tttatagtag tgtgagaatg gactaataca 120
agtacatttt acttagtaat aataataaac aaatatatta catttttgtg tatttactac 180
accatatttt ttattgttat tgtagtgtac accttctact tattaaaaga aataggcccg 240
aggcgggcag atcacgaggt caggagatgg agaccactac gtcgatac 288

<210> 197
<211> 289
<212> DNA
<213> Homo sapien

<400> 197
ttgggcacct tcaatatcat gacaggtgat gtgataacca agaaggctac taagtgatta 60
atgggtgggt aatgtataca gagtaggtac actggacaga ggggtaattc atagccaagg 120
caggagaagc agaatggcaa aacatttcat cacactactc aggatagcat gcagtttaaa 180
acctataagt agtttatttt tggaattttc cacttaatat tttcagactg caggtaacta 240
aactgtggaa cacaagaaca tagataaggg gagaccacta cgtcgatac 289

<210> 198
<211> 288
<212> DNA
<213> Homo sapien

<400> 198
gtatcgacgt agtggtctcc caagcagtgg gaagaaaacg tgaaccaatt aaaatgtatc 60
agatacccca aagaaaggcg cttgagtaaa gattccaagt gggtcacaat ctcagatctt 120
aaaattcagg ctgtcaaaga gatttgctat gaggttgctc tcaatgactt caggcacagt 180
cggcaggaga ttgaagccct ggccattgtc aagatgaagg agctttgtgc catgtatggc 240
aagaaagacc ccaatgagcg ggactcctgg agaccactac gtcgatac 288

<210> 199
<211> 1027
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(1027)
<223> n = A,T,C or G

<400> 199
gctttttggg aaaaacncaa ntgggggaaa gggggnttnn tngcaagggg ataaaggggg 60

aancccaggg tttccccatt cagggaggtg taaaaagncg gccaggggat tgtaanagga 120
ttcaataata gggggaatgg gcccngaagt tgcaaggttc cngcccgcca tgnccgcggg 180
atttagtgac attacgacgs tggtaataaa gtgggsccaa waaatatttg tgatgtgatt 240
tttsgaccag tgaacccatt gwacaggacc tcatttccty tgagatgrta gccataatca 300
gataaaagrt tagaagtytt tctgcacgtt aacagcatca ttaaatggag tggcatcacc 360
aatttcaccc tttgttagcc gataccttcc ccttgaaggc attcaattaa gtgaccaatc 420
gtcatacgag aggggatggc atggggattg atgatgatat caggggtgat accttcacag 480
gtgaaaggca tatcctcttg tctatactga ataccacaag tacccttttg accatgtcga 540
ctagcaaatt tgtctccaat ctgtgtwatc cctaacagag cgtaccctta ttttacaaaa 600
tttatatcct tcctgattga gagttaccat aacctgatcc acaatgcccg tctcgctwgt 660
tctgagaaaa gtgctacagt ctctcttggt atagcgtcta ttggtgctct ccaattcatc 720
ttcatttttc aggcaaggtg aactgttttg cctataataa cmtcatctcc tgatacmcga 780
aacccckgga rctatcaaac catcatcatc cagcgttckt watgtymcta aatccctatt 840
gcggccgcct gcaggtcaac atatnggaaa acccccccacc ccttnggagc ntaccttgaa 900
ttttccatat gtcccntaaa ttanctngnc ttancctggc cntaacctnt tccggtttaa 960
attgtttccg cccccnttcc ccnccttnna accggaaacc ttaattttna accnggggtt 1020
cctatcc 1027

<210> 200
<211> 207
<212> DNA
<213> Homo sapien

<400> 200
agtgacatta cgacgctggc catcttgaat cctagggcat gaagttgccc caaagttcag 60
cacttggtta agcctgatcc ctctggttta tcacaaagaa taggatggga taaagaaagt 120
ggacacttaa ataagctata aattatatgg tccttgtcta gcaggagaca actgcacagg 180
tatactacca gcgtcgtaat gtcacta 207

<210> 201
<211> 209
<212> DNA
<213> Homo sapien

<400> 201
tgggcacctt caatatctat taaaagcaca aatactgaag aacacaccaa gactatcaat 60
gaggttacat ctggagtcct cgatatatca ggaaaaaatg aagtgaacat tcacagagtt 120
ttacttcttt gggaactcaa atgctagaaa agaaaagggt gccctctttc tctggcttcc 180
tggtcctatc cagcgtcgta atgtcacta 209

<210> 202
<211> 349
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(349)
<223> n = A,T,C or G

<400> 202

ntacgctgca acactgtgga gccactggtt tttattcccg gcaggttatc cagcaaacag 60
tcactgaaca caccgaagac cgtggtatgg taaccgttca cagtaatcgt tccagtcgtc 120
tgcgggaccc cgacgagcgt cactgggtac agaccagatt cagccggaag agaaagcgcc 180
gcagggagag actcgaactc cactccgctg gtgagcagcc ccatgttttc aactcgaagt 240
tcaaacggca ttgggttata taccatcagc tgaacttcac acacatctcc ttgaacccac 300
tggaaatcta ttttcttgtt ccgctcttct ccacagtgtt gcagcgtaa 349

<210> 203
<211> 241
<212> DNA
<213> Homo sapien

<400> 203
tgctcctctt gccttaccaa cccaaagccc actgtgaaat atgaagtgaa tgacaaaatt 60
cagttttcaa cgcaatatag tatagtttat ctgattcttt tgatctccag gacactttaa 120
acaactgcta ccaccaccac caacctaggg atttaggatt ctccacagac cagaaattat 180
ttctcctttg agtttcaggc tcctctggga ctcctgttca tcaatgggtg gtaaatggct 240
a 241

<210> 204
<211> 248
<212> DNA
<213> Homo sapien

<400> 204
tagccattta ccacccatct gcaaaccswg acmwwcargr cywgwackya ggcgatttga 60
agtactggta atgctctgat catgttagtt acataagtgt ggtcagttta caaaaattca 120
cagaactaaa tactcaatgc tatgtgttca tgtctgtgtt tatgtgtgtg taatgtttca 180
attaagtttt tttaaaaaaa agagatgatt tccaaataag aaagccgtgt tggtaaggca 240
agaggagc 248

<210> 205
<211> 505
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(505)
<223> n = A,T,C or G

<400> 205
tacgctgcaa cactgtggag ccattcatac aggtccctaa ttaaggaaca agtgattatg 60
ctacctttgc acggttaggg taccgcggcc gttaaacatg tgtcactggg caggcggtgc 120
ctctaatact ggtgatgcta gaggtgatgt ttttggtaaa caggcggggt aagatttgcc 180
gagttccttt tacttttttt aacctttcct tatgagcatg cctgtgttgg gttgacagtg 240
ggggtaataa tgacttgttg gttgattgta gatattgggc tgttaattgt cagttcagtg 300
ttttaatctg acgcaggctt atgcggagga gaatgttttc atgttactta tactaacatt 360
agttcttcta tagggtgata gattggtcca attgggtgtg aggagttcag ttatatgttt 420
gggatttttt aggtagtggg tgttganctt gaacgctttc ttaattggtg gctgctttta 480
rgcctactat gggtggtaaa tggct 505

<210> 206
<211> 179
<212> DNA
<213> Homo sapien

<400> 206
tagactgact catgtcccct accaaagccc atgtaaggag ctgagttctt aaagactgaa 60
gacagactat tctctggaga aaaataaaat ggaaattgta ctttaaaaaa aaaaaaaatc 120
ggccgggcat ggtagcacac acctgtaatc ccagctacta ggggacatga gtcagtcta 179

<210> 207
<211> 176
<212> DNA
<213> Homo sapien

<400> 207
agactgactc atgtccccta ccccaccttc tgctgtgctg ccgtgttcct aacaggtcac 60
agactggtac tggtcagtgg cctgggggtt ggggacctct attatatggg atacaaattt 120
aggagttgga attgacacga tttagtgact gatgggatat gggtggtaaa tggcta 176

<210> 208
<211> 196
<212> DNA
<213> Homo sapien

<400> 208
agactgactc atgtccccta tttaacaggg tctctagtgc tgtgaaaaaa aaaaatgctg 60
aacattgcat ataacttata ttgtaagaaa tactgtacaa tgactttatt gcatctgggt 120
agctgtaagg catgaaggat gccaagaagt ttaaggaata tgggtggtaa atggctaggg 180
gacatgagtc agtcta 196

<210> 209
<211> 345
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(345)
<223> n = A,T,C or G

<400> 209
gacgcttggc cacttgacac cttttatttt ttaaggattc ttaagtcatt tangtnactt 60
tgtaagtttt tcctgtgccc ccataagaat gatagcttta aaaattatgc tggggtagca 120
aagaagatac ttctagcttt agaatgtgta ggtatagcca ggattcttgt gaggaggggt 180
gatttagagc aaatttctta ttctccttgc ctcatctgta acatggggat aataatagaa 240
ctggcttgac aaggttggaa ttagtattac atggtaaata catgtaaaat gtttagaatg 300
gtgccaagta tctaggaagt acttgggcat gggtggtaaa tggct 345

<210> 210

<211> 178
<212> DNA
<213> Homo sapien

<400> 210
gacgcttggc cacttgacac tagagtaggg tttggccaac tttttctata aaggaccaga 60
gagtaaatat ttcaggcttt gtgggttgtg cagtctctct tgcaactact cagctctgcc 120
attgtagcat agaaatcagc catagacagg acagaaatga atgggtggta aatggcta 178

<210> 211
<211> 454
<212> DNA
<213> Homo sapien

<400> 211
tgggcacctt caatatctat ccagcgcatc taaattcgct tttttcttga ttaaaaattt 60
caccacttgc tgtttttgct catgtatacc aagtagcagt ggtgtgaggc catgcttgtt 120
ttttgattcg atatcagcac cgtataagag cagtgctttg gccattaatt tatcttcatt 180
gtagacagca tagtgtagag tggtatctcc atactcatct ggaatatttg gatcagtgcc 240
atgttccagc aacattaacg cacattcatc ttcctggcat tgtacggcct ttgtcagagc 300
tgtcctcttt ttgttgtcaa ggacattaag ttgacatcgt ctgtccagca cgagttttac 360
tacttctgaa ttcccattgg cagaggccag atgtagagca gtcctctttt gcttgtccct 420
cttgttcaca tcagtgtccc tgagcataac ggaa 454

<210> 212
<211> 337
<212> DNA
<213> Homo sapien

<400> 212
tccgttatgc cacccagaaa acctactgga gttacttatt aacatcaagg ctggaaccta 60
tttgcctcag tcctatctga ttcatgagca catggttatt actgatcgca ttgaaaacat 120
tgatcacctg ggtttcttta tttatcgact gtgtcatgac aaggaaactt acaaactgca 180
acgcagagaa actattaaag gtattcagaa acgtgaagcc agcaattgtt tcgcaattcg 240
gcattttgaa aacaaatttg ccgtggaaac tttaatttgt tcttgaacag tcaagaaaaa 300
cattattgag gaaaattaat atcacagcat aacggaa 337

<210> 213
<211> 715
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(715)
<223> n = A,T,C or G

<400> 213
tcgggtgatg cctcctcagg catcttccat ccatctcttc aagattagct gtcccaaatg 60
tttttccttc tcttctttac tgataaattt ggactccttc ttgacactga tgacagcttt 120
agtatccttc ttgtcacctt gcagacttta aacataaaaa tactcattgg ttttaaaagg 180

aaaaaagtat acattagcac tattaagctt ggccttgaaa cattttctat cttttattaa 240
atgtcggtta gctgaacaga attcatttta caatgcagag tgagaaaaga agggagctat 300
atgcatttga gaatgcaagc attgtcaaat aaacatttta aatgctttct taaagtgagc 360
acatacagaa atacattaag atattagaaa gtgtttttgc ttgtgtacta ctaattaggg 420
aagcaccttg tatagttcct cttctaaaat tgaagtagat tttaaaaacc catgtaattt 480
aattgagctc tcagttcaga ttttaggaga attttaacag ggatttggtt ttgtctaaat 540
tttgtcaatt tntttagtta atctgtataa ttttataaat gtcaaactgt atttagtccg 600
ttttcatgct gctatgaaag aaatacccan gacagggtta tttataaang gaaagangtt 660
aatttgactc ccagttcaca ggcctgagga ngnatcnccc gaaatcctta ttgcg 715

<210> 214
<211> 345
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(345)
<223> n = A,T,C or G

<400> 214
ggtaangngc atacntcggt gctccggccg ccggagtcgg gggattcggg tgatgcctcc 60
tcaggcccac ttgggcctgc ttttcccaaa tggcagctcc tctggacatg ccattccttc 120
tcccacctgc ctgattcttc atatgttggg tgtccctgtt tttctggtgc tatttcctga 180
ctgctgttca gctgccactg tcctgcaaag cctgcctttt taaatgcctc accattcctt 240
catttgtttc ttaaatatgg gaagtgaaag tgccacctga ggccgggcac agtggctcac 300
gcctgtaatc ccagcacttt gggagcctga ggaggcatca cccga 345

<210> 215
<211> 429
<212> DNA
<213> Homo sapien

<400> 215
ggtgatgcct cctcaggcga agctcaggga ggacagaaac ctcccgtgga gcagaagggc 60
aaaagctcgc ttgatcttga ttttcagtac gaatacagac cgtgaaagcg gggcctcacg 120
atccttctga ccttttgggt tttaagcagg aggtgtcaga aaagttacca cagggataac 180
tggcttgtgg cggccaagcg ttcatagcga cgtcgctttt tgatccttcg atgtcggctc 240
ttcctatcat tgtgaagcag aattcaccaa gcgttggatt gttcacccac taatagggaa 300
cgtgagctgg gtttagaccg tcgtgagaca ggttagtttt accctactga tgatgtgtkg 360
ttgccatggt aatcctgctc agtacgagag gaaccgcagg ttcasacatt tggtgtatgt 420
gcttgcctt 429

<210> 216
<211> 593
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(593)

<223> n = A,T,C or G

<400> 216
tgacacctat gtccngcatc tgttcacagt ttccacaaat agccagcctt tggccacctc 60
tctgtcctga ggtatacaag tatatcagga ggtgtatacc ttctcttctc ttccccacca 120
aagagaacat gcaggctctg gaagctgtct taggagcctt tgggctcaga atttcagagt 180
cttgggtacc ttggatgtgg tctggaagga gaaacattgg ctctggataa ggagtacagc 240
cggaggaggg tcacagagcc ctcagctcaa gcccctgtgc cttagtctaa aagcagcttt 300
ggatgaggaa gcaggttaag taacatacgt aagcgtacac aggtagaaag tgctgggagt 360
cagaattgca cagtgtgtag gagtagtacc tcaatcaatg agggcaaatc aactgaaaga 420
agaagaccna ttaatgaatt gcttangggg aaggatcaag gctatcatgg agatctttct 480
aggaagatta ttgtttanaa ttatgaaagg antagggcag ggacagggcc agaagtanaa 540
ganaacattg cctatanccc ttgtcttgca cccagatgct ggacaaggtg tca 593

<210> 217
<211> 335
<212> DNA
<213> Homo sapien

<400> 217
tgacaccttg tccagcatct gacgtgaaga tgagcagctc agaggaggtg tcctggattt 60
cctggttctg tgggctccgt ggcaatgaat tcttctgtga agtggatgaa gactacatcc 120
aggacaaatt taatcttact ggactcaatg agcaggtccc tcactatcga caagctctag 180
acatgatctt ggacctggag cctgatgaag aactggaaga caaccccaac cagagtgacc 240
tgattgagca ggcagccgag atgctttatg gattgatcca cgcccgctac atccttacca 300
accgtggcat cgcccagatg ctggacaagg tgtca 335

<210> 218
<211> 248
<212> DNA
<213> Homo sapien

<400> 218
tacgtactgg tcttgaaggt cttaggtaga gaaaaaatgt gaatatttaa tcaaagacta 60
tgtatgaaat gggactgtaa gtacagaggg aagggtggcc cttatcgcca gaagttggta 120
gatgcgtccc cgtcatgaaa tgttgtgtca ctgcccgaca tttgccgaat tactgaaatt 180
ccgtagaatt agtgcaaatt ctaacgttgt tcatctaaga ttatggttcc atgtttctag 240
tactttta 248

<210> 219
<211> 530
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(530)
<223> n = A,T,C or G

<400> 219
tgacgcttgg ccacttgaca caagtagggg ataaggacaa agacccatna ggtggcctgt 60

cagccttttg ttactgttgc ttccctgtca ccacggcccc ctctgtaggg gtgtgctgtg 120
ctctgtggac attggtgcat tttcacacat accattctct ttctgcttca cagcagtcct 180
gaggcgggag cacacaggac taccttgtca gatgangata atgatgtctg gccaactcac 240
cccccaacct tctcactagt tatangaaga gccangccta naaccttcta tcctgncccc 300
ttgccctatg acctcatccc tgttccatgc cctattctga tttctggtga actttggagc 360
agcctggttt ntcctcctca ctccagcctc tctccatacc atggtanggg ggtgctgttc 420
cacncaaang gtcaggtgtg tctggggaat cctnananct gccnggagtt tccnangcat 480
tcttaaaaac cttcttgcct aatcanatng tgtccagtgg ccaaccntcn 530

<210> 220
<211> 531
<212> DNA
<213> Homo sapien

<400> 220
tgacgcttgg ccacttgaca ctaaatagca tcttctaaag gcctgattca gagttgtgga 60
aaattctccc agtgtcaggg attgtcagga acagggctgc tcctgtgctc actttacctg 120
ctgtgtttct gctggaaaag gagggaagag gaatggctga tttttaccta atgtctccca 180
gtttttcata ttcttcttgg atcctcttct ctgacaactg ttccctttttg gtcttcttct 240
tcttgctcag agagcaggtc tctttaaaac tgagaaggga gaatgagcaa atgattaaag 300
aaaacacact tctgaggccc agagatcaaa tattaggtaa atactaaacc gcttgcctgc 360
tgtggtcact tttctcctct ttcacatgct ctatccctct atcccccacc tattcatatg 420
gcttttatct gccaagttat ccggcctctc atcaaccttc tcccctagcc tactggggga 480
tatccatctg ggtctgtctc tggtgtattg gtgtcaagtg gccaagcgtc a 531

<210> 221
<211> 530
<212> DNA
<213> Homo sapien

<400> 221
attgacgctt ggccacttga cacccgcctg cctgcaatac tggggcaagg gccttcactg 60
ctttcctgcc accagctgcc actgcacaca gagatcagaa atgctaccaa ccaagactgt 120
tggtcctcag cctctctgag gagaaagagc agaagcctgg aagtcagaag agaagctaga 180
tcggctacgg ccttggcagc cagcttcccc acctgtggca ataaagtcgt gcatggctta 240
acaatggggg cacctcctga gaaacacatt gttaggcaat tcggcgtgtg ttcatcagag 300
catatttaca caaacctcga tagtgcagcc tactatccac tattgctcct acgctgcaaa 360
cctgaacagc atgggactgt actgaatact ggaagcagct ggtgatggta cttatttgtg 420
tatctaaaca cagagaaggt acagtaagaa tatggtatca taaacttaca gggaccgcca 480
tcctatatgc agtctgttgt gaccaaaatg tgtcaagtgg ccaagcgtca 530

<210> 222
<211> 578
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(578)
<223> n = A,T,C or G

<400> 222
tgtatcgacg tagtggtctc cgggctacta ggccgttgtg tgctggtagt acctggttca 60
ctgaaaggcg catctccctc cccgcgtcgc cctgaagcag ggggaggact tcgcccagcc 120
aaggcagttg tatgagtttt agctgcggca cttcgagacc tctgagccca cctccttcag 180
gagccttccc cgattaagga agccagggta aggattcctt cctcccccag acaccacgaa 240
caaaccacca ccccccctat tctggcagcc catatacatc agaacgaaac aaaaataaca 300
aataaacnaa aaccaaaaaa aaaagagaag gggaaatgta tatgtctgtc catcctgttg 360
ctttagcctg tcagctccta nagggcaggg accgtgtctt ccgaatggtc tgtgcagcgc 420
cgactgcggg aagtatcgga ggaggaagca gagtcagcag aagttgaacg gtgggcccgg 480
cggctcttgg gggctggtgt tgtacttcga gaccgctttc gctttttgtc ttagatttac 540
gtttgctctt tggagtggga naccactacn tcnataca 578

<210> 223
<211> 578
<212> DNA
<213> Homo sapien

<400> 223
tgtatcgacg tagtggtctc ctcttgcaaa ggactggctg gtgaatggtt tccctgaatt 60
atggacttac cctaaacata tcttatcatc attaccagtt gcaaaatatt agaatgtgtt 120
gtcactgttt catttgattc ctagaaggtt agtcttagat atgttacttt aacctgtatg 180
ctgtagtgct ttgaatgcat tttttgtttg catttttgtt tgcccaacct gtcaattata 240
gctgcttagg tctggactgt cctggataaa gctgttaaaa tattcaccag tccagccatc 300
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ctcaatttct gggttcattt tgggtgccct aaatcttagg gtgtgacttt cttagcatcc 420
tgtaacatcc attcccaagc aagcacaact tcacataata ctttccagaa gttcattgct 480
gaagcctttc cttcacccag cggagcaact tgattttcta caacttccct catcagagcc 540
acaagagtat gggatatgga gaccactacg tcgataca 578

<210> 224
<211> 345
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(345)
<223> n = A,T,C or G

<400> 224
tgtatcgacg tantggtctc ccaaggtgct gggattgcag gcatgagcca ccactcccag 60
gtggatcttt ttctttatac ttacttcatt aggtttctgt tattcaagaa gtgtagtggt 120
aaaagtcttt tcaatctaca tggttaaata atgatagcct gggaaataaa tagaaatttt 180
ttctttcatc tttaggttga ataaagaaac agaaaaaata gaacatactg aaaataatct 240
aagttccaac catagaagaa ctgcagaaga aatgaagaaa gtgatgatga tttagatttt 300
gatattgatt tagaagacac aggaggagac cactacgtcg ataca 345

<210> 225
<211> 347
<212> DNA
<213> Homo sapien

<400> 225
tgtatcgacg tagtggtctc caaactgagg tatgtgtgcc actagcacac aaagccttcc 60
aacagggacg caggcacagg cagtttaaag ggaatctgtt tctaaattaa tttccacctt 120
ctctaagtat tctttcctaa aactgatcaa ggtgtgaagc ctgtgctctt tcccaactcc 180
cctttgacaa cagccttcaa ctaacacaag aaaaggcatg tctgacactc ttcctgagtc 240
tgactctgat acgttgttct gatgtctaaa gagctccaga acaccaaagg gacaattcag 300
aatgctggtg tataacagac tccaatggag accactacgt cgataca 347

<210> 226
<211> 281
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(281)
<223> n = A,T,C or G

<400> 226
aggngnggga ntgtatcgac gtagtggtct cccaacagtc tgtcattcag tctgcaggtg 60
tcagtgtttt ggacaatgag gcaccattgt cacttattga ctcctcagct ctaaatgctg 120
aaattaaatc ttgtcatgac aagtctggaa ttcctgatga ggttttacaa agtattttgg 180
atcaatactc caacaaatca gaaagccaga aagaggatcc tttcaatatt gcagaaccac 240
gagtggattt acacacctca ggagaccact acgtcgatac a 281

<210> 227
<211> 3646
<212> DNA
<213> Homo sapien

<400> 227
gggaaacact tcctcccagc cttgtaaggg ttggagccct ctccagtata tgctgcagaa 60
tttttctctc ggtttctcag aggattatgg agtccgcctt aaaaaaggca agctctggac 120
actctgcaaa gtagaatggc caaagtttgg agttgagtgg ccccttgaag ggtcactgaa 180
cctcacaatt gttcaagctg tgtggcgggt tgttactgaa actcccggcc tccctgatca 240
gtttccctac attgatcaat ggctgagttt ggtcaggagc accccttccg tggctccact 300
catgcaccat tcataatttt acctccaagg tcctcctgag ccagaccgtg ttttcgcctc 360
gaccctcagc cggttcggct cgccctgtac tgcctctctc tgaagaagag gagagtctcc 420
ctcacccagt cccaccgcct taaaaccagc ctactccctt agggtcatcc catgtctcct 480
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aatcctccct tctctgaaaa gccccaggct ttgacctcac tgatggagtc tgtactctgg 720
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cccagatagt aaaaggaaac tccaaaaact agagggattt tgctggaatg aataccagtc    1200
agcttttaga gatagcctaa aaggtttttg acagtcaaga ggttgaaaaa caaaaacaag    1260
cagctcaggc agctgaaaaa agccactgat aaagcatcct ggagtatcag agtttactgt    1320
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ggtaaccaga aagctgattc agcagctcaa gatgcagtgt gactttcagt cacgcctcta    1860
aacttgctgc ccacagtctc ctttccacag ccagatctgc ctgacaatcc cgcatactca    1920
acagaagaag aaaactggcc tcagaactca gagccaataa aaatcaggaa ggttggtgga    1980
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aggtaaatgc caaaaaaggt cctaaaccca gcccaggcca ccgtctccaa gaaaactcac    2220
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tgggctgggt tcttaccttt tgaaatcatg tatgggaggg tgctgcctat cttgcctaag    2700
ctaagagatg cccaattggc aaaaatatca caaactaatt tattacagta cctacagtct    2760
ccccaacagg tacaagatat catcctgcca cttgttcgag gaacccatcc caatccaatt    2820
cctgaacaga cagggccctg ccattcattc ccgccaggtg acctgttgtt tgttaaaaag    2880
ttccagagag aaggactccc tcctgcttgg aagagacctc acaccgtcat cacgatgcca    2940
acggctctga aggtggatgg cattcctgcg tggattcatc actcccgcat caaaaaggcc    3000
aacagagccc aactagaaac atgggtcccc agggctgggt caggcccctt aaaactgcac    3060
ctaagttggg tgaagccatt agattaattc tttttcttaa ttttgtaaaa caatgcatag    3120
cttctgtcaa acttatgtat cttaagactc aatataaccc ccttgttata actgaggaat    3180
caatgatttg attcccccaa aaacacaagt ggggaatgta gtgtccaacc tggtttttac    3240
taaccctgtt tttagactct ccctttcctt taatcactca gcttgtttcc acctgaattg    3300
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ttcctcaagg acttaacttg tgcaagctga ctcccagcac atccaagaat gcaattaact    3420
gataagatac tgtggcaagc tatatccgca gttcccagga attcgtccaa ttgatcacag    3480
cccctctacc cttcagcaac caccaccctg atcagtcagc agccatcagc accgaggcaa    3540
ggccctccac cagcaaaaag attctgactc actgaagact tggatgatca ttagtatttt    3600
tagcagtaaa gttttttttt cttttctttt cttttttcct cgtgcc                   3646
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<210> 228
<211> 419
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(419)

<223> n = A,T,C or G

<400> 228
taagagggta caagatctaa gcacagccgt caatgcagaa cacagaacgt agcctggtaa 60
gtgtgttaag agtgggaatt tttggagtac agagtaaggc acctaaccct agctggggtt 120
tggtgacggt cccagatggc ttacagaaga aagtgtcctg agatgagttt ttaagaatga 180
ataaggatag acacaagtga ggactgactt ggcagtggtg aatggtgggt ggcaaaaaac 240
ttcgcatgta tggaaactgc acgtacagga atgaagaatg agactgtgtg gtgtttaatg 300
agctgcaaat actaatttta tcctgaaagt tttgaagagt taactaaaaa gtatttttta 360
gtaaggaaat aaccctacat ttcagggtta ttgtttgttt anatattgaa ggtgcccaa 419

<210> 229
<211> 148
<212> DNA
<213> Homo sapien

<400> 229
aagagggtac ctgtatgtag ccatggtggc aatgagagac tgattactac ctgctggaga 60
ttgtttaagt gagttaatat attaaggata aagggagcca ggtttttttga ctgttggaga 120
aggaaattac agatattgaa ggtcccaa 148

<210> 230
<211> 257
<212> DNA
<213> Homo sapien

<400> 230
taagagggta cmaaaaaaaa aaaatagaac gaatgagtaa gacctactat ttgatagtac 60
aacagggtga ctatagtcaa tgataactta attatacatt taacatagag tgtaattgga 120
ttgtttgtaa ctcgaaggat aaatgcttga gaggatggat accccattct ccatgatgta 180
cttatttcac attacatgcc tgtatcaaag catctcatat accctataaa tatgtacacc 240
tactatgtac cctctta 257

<210> 231
<211> 260
<212> DNA
<213> Homo sapien

<400> 231
taagagggta cgggtatttg ctgatgggat ttttttttct ttctttttct ttggaaaaca 60
aaatgaaagc cagaacaaaa ttattgaaca aaagacaggg actaaatctg gagaaatgaa 120
gtcccctcac ctgactgcca tttcattcta tctgaccttc cagtctaggt taggagaata 180
gggggtggag gggattaatc tgatacaggt atatttaaag caactctgca tgtgtgccag 240
aagtccatgg taccctctta 260

<210> 232
<211> 596
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(596)
<223> n = A,T,C or G

<400> 232
tgctcctctt gccttaccaa ccacaaatta gaaccataat gagatgtcac ctcatacctg 60
gtgggattaa cattatttaa aaaatcagaa gtattgacaa ggatgtgaag aaattagaac 120
atctgtgcac tgttggtggg aatgtaaaaa aggtgtggcc actatgggta acagcatgaa 180
ggttcctcaa aaaaaatttt ttttaatcta ctctatgatc gatcttgagg ttgtttatgc 240
aaaagaactg aaatcaggat tttgaggaaa tattcacatt cccacatcca tttctgcttt 300
attcataata ctcaagagat ggaaacaacc taaatgtcca tcccgggatg aatggataaa 360
cacagtgtgg tatatgcata caatggaata ttatttagtc tttaaaaaga aaaattctat 420
catatactac aacttanatn aaccttgagg acacaatgct nagtgaaata agccacggaa 480
ggacgaatac tgcattattc ccttatatga agtatctaaa gtggtcaaac tcttanagca 540
naaagtaaaa atgggtggtt gccanacagt tggttaggcn agaaganaan cctant 596

<210> 233
<211> 96
<212> DNA
<213> Homo sapien

<400> 233
tcttctgaag acctttcgcg actcttaagc tcgtggttgg taaggcaaga ggagcgttgg 60
taaggcaaga ggagcgttgg taaggcaaga ggagca 96

<210> 234
<211> 313
<212> DNA
<213> Homo sapien

<400> 234
tgtaagtcga gcagtgtgat gataaaactt gaatggatca atagttgctt cttatggatg 60
agcaaagaaa gtagtttctt gtgatggaat ctgctcctgg caaaaatgct gtgaacgttg 120
ttgaaaagac aacaaagagt ttagagtagt acataaattt agaatagtac ataaacttag 180
aatagtacat aaacttagta cataaataat gcacgaagca ggggcagggc ttgagagaat 240
tgacttcaat ttggaaagag tatctactgt aggttagatg ctctcaaaca gcatcacact 300
gctcgactta caa 313

<210> 235
<211> 550
<212> DNA
<213> Homo sapien

<400> 235
aacgaggaca gatccttaaa aagaatgttg agtgaaaaaa gtagaaaata agataatctc 60
caaagtccag tagcattatt taaacatttt taaaaaatac actgataaaa attttgtaca 120
tttcccaaaa atacatatgg aagcacagca gcatgaatgc ctatgggrtt gaggataggg 180
gttgggagta gggatgggga taaaggggga aaataaaacc agagaggagt cttacacatt 240
tcatgaacca aggagtataa ttatttcaac tatttgtacc wgaagtccag aaagagtgga 300
ggcagaaggg ggagaagagg gcgaagaaac gtttttggga gaggggtccc asaagagaga 360
ttttcgcgat gtggcgctac atacgttttt ccaggatgcc ttaagctctg caccctattt 420

ttctcatcac taatattaga ttaaaccctt tgaagacagc gtctgtggtt tctctacttc 480
agctttccct ccgtgtcttg cacacagtag ctgttttaca agggttgaac tgactgaagt 540
gagattattc 550

<210> 236
<211> 325
<212> DNA
<213> Homo sapien

<400> 236
tagactgact catgtcccct accagagtag ctagaattaa tagcacaagc ctctacaccc 60
aggaactcac tattgaatac ataaatggaa tttattcagc cttaaaaagt ttggaaggaa 120
attctgacat atgctaaaac atggatgaac cttgaagact ttatgataag taaaagaagc 180
cagtcataaa aggaaaaata ttgcatgatt ccacttatat gaggtaccta gagtagtcaa 240
tttcatagaa acacaaaata gaatggtgtt tgccagggct tttgaggaaa agggaatgac 300
aagttagggg acatgagtca gtcta 325

<210> 237
<211> 373
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(373)
<223> n = A,T,C or G

<400> 237
tagactgact catgtcccct atctactcaa catttccact tgaagtctga taggcatctc 60
agacttatct tgtcccaaag caaactcttt atttcttttc atcctagtct ttatttcttg 120
tgctgtctta cccatctcaa aagagtgcca aaatccacca agttgctgaa acagaaatct 180
aagaaatatc cttgattctt ctttttccca tctacttcac ttctaattca ttagtaaata 240
atctgtttca gaaaaccaaa cacctcatgt tctcactcat aaggggggagt tgaacaatga 300
gaacacacag acacagggag gggaacatca cacaccacgg cccgtcaggg agtangggac 360
atgagtcagt cta 373

<210> 238
<211> 492
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(492)
<223> n = A,T,C or G

<400> 238
tagactgact catgtcccct ataatgctcc caggcatcag aaagcatctc aaactggagc 60
tgacaccatg gcagaggttt caggtaagtc acaaaagggg tcctaaagaa tttgccctca 120
atatcagagt gattagaaga agtggacaga gctacccaag ttaaacatat gcgagataaa 180
aaaaatatgg cacttgtgaa cacacactac aggaggaaaa taaggaacat aatagcatat 240

tgtgctatta tgatgatgaa gaacctctct anaagaaaac ataaccaaag aaacaaagaa 300
aattcctgcn aatgtttaat gctatagaag aaattaacaa aaacatatat tcaatgaatt 360
cagaaaagtt agcaggtcan aagaaaacaa atcaaagacc agaataatcc cattttagat 420
tgtcgagtaa actanaacag aaagaatacc actggaaatt gaattcctac gtangggaca 480
tgantcantc ta 492

<210> 239
<211> 482
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(482)
<223> n = A,T,C or G

<400> 239
tggaaagtat ttaatgatgg gcaacttgct gtttacttcc tacatatccc atcatcttct 60
gtatttttt aaataacttt tttttggatt tttaaagtaa ccttattctg agaggtaaca 120
tggattacat acttctaagc cattaggaga ctctatgtta aaccaaaagg aaatgttact 180
agatcttcat ttgatcaata ggatgtgata atcatcatct ttctgctcta atggaaaagt 240
actanaaaca tggaaccata atcttagatg aacaacgtta gaatttgcac taattctacg 300
gaatttcagt aattcggcaa atgtcgggca gtgacacaac atttcatgac ggggacgcat 360
ctaccaactt ctggcgataa gggccaccct tccctctgta cttacagtcc catttcatac 420
acagtctttg attaaatatt cacatttttt ctctacctaa agaccttcaa gaccagtacg 480
ta 482

<210> 240
<211> 519
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(519)
<223> n = A,T,C or G

<400> 240
tgtatcgacg tagtggtctc cccatgtgat agtctgaaat atagcctcat gggatgagag 60
gctgtgcccc agcccgacac ccgtaaaggg tctgtgctga ggtggattag taaaagagga 120
aagccttgca gttgagatag aggaagggca ctgtctcctg cctgcccctg ggaactgaat 180
gtctcggtat aaaacccgat tgtacatttg ttcaattctg agataggaga aaaaccaccc 240
tatggcggga ggcgagacat gttggcagca atgctgcctt gttatgcttt actccacaga 300
tgtttgggcg gagggaaaca taaatctggc ctacgtgcac atccaggcat agtacctccc 360
tttgaactta attatgacac agattccttt gctcacatgt ttttttgctg accttctcct 420
tattatcacc ctgctctcct accgcattcc ttgtgctgag ataatgaaaa taatatcaat 480
aaaaacttga nggaactcgg agaccactac gtcgataca 519

<210> 241
<211> 771
<212> DNA

<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(771)
<223> n = A,T,C or G

<400> 241
tgtatcgacg tagtggtctc cactcccgcc ttgacggggc tgctatctgc cttccaggcc 60
actgtcacgg ctcccgggta gaagtcactt atgagacaca ccagtgtggc cttgttggct 120
tgaagctcct cagaggaggg tgggaacaga gtgaccgagg gggcagcctt gggctgacct 180
aggacggtca gcttggtccc tccgccaaac acgagagtgc tgctgcttgt atatgagctg 240
cagtaataat cagcctcgtc ctcagcctgg agcccagaga tggtcaggga ggccgtgttg 300
ccanacttgg agccagagaa gcgattagaa acccctgagg gccgattacc gacctcataa 360
atcatgaatt tgggggcttt gcctgggtgc tgttggtacc angagacatt attataacca 420
ccaacgtcac tgctggttcc antgcaggga aaatggttga tcnaactgtc caagaaaacc 480
actacgtcca taccaatcca ctaattgccn gccgcctgca ggttcaacca tattggggaa 540
naactccccn ccgccgtttg ggattgncat naacctttga aatttttcc tattanttgt 600
ccccctaaaa taaaccnttg ggcnttaatc cattgggtcc atancttntt tncccggttt 660
ttaaaanttg tttatcccgc cncccnattt ccccccccaac tttccaaaac ccgaaaccnt 720
tnaaatttnt tnaaaccctg gggggttccc nnaattnnan ttnaanctnc c 771

<210> 242
<211> 167
<212> DNA
<213> Homo sapien

<400> 242
tgggcacctt caatatcggg ctcatcgata acatcacgct gctgatgctg ctgttgctgg 60
tcctctctag gaacctctgg attttcaaat tctttgagga attcatccaa attatctgcc 120
tctcctcctt tcctcctttt tctaaggtct tctggtacaa gcggtca 167

<210> 243
<211> 338
<212> DNA
<213> Homo sapien

<400> 243
ttgggcacct tcaatatcta ctgatctaaa tagtgtggtt tgaggcctct tgttcctggc 60
taaaaatcct tggcaagagt caatctccac tttacaatag aggtaaaaat cttacaatgg 120
atattcttga caaagctagc atagagacag caattttaca caaggtattt ttcacctgtt 180
taataacagt ggttttccta cacccatagg gtgccaccaa gggaggagtg cacagttgca 240
gaaacaaatt aagatactga agacaacact acttaccatt tcccgtatag ctaaccacca 300
gttcaactgt acatgtatgt tcttatgggc aatcaaga 338

<210> 244
<211> 346
<212> DNA
<213> Homo sapien

<400> 244

tttttggctc ccatacagca cactctcatg ggaaatgtct gttctaaggt caacccataa 60
tgcaaaaatc atcaatatac ttgaagatcc ccgtgtaagg tacaatgtat ttaatattat 120
cactgataca attgatccaa taccagtttt agtctggcat tgaatcaaat cactgttttt 180
gttgtataaa aagagaaata tttagcttat atttaagtac catattgtaa gaaaaaagat 240
gcttatcttt acatgctaaa atcatgatct gtacattggt gcagtgaata ttactgtaaa 300
agggaagaag gaatgaagac gagctaagga tattgaaggt gcccaa 346

<210> 245
<211> 521
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(521)
<223> n = A,T,C or G

<400> 245
accaatccca cacggatact gagggacaag tatatcatcc catttcatcc ctacagcagc 60
aacttcatga ggcaggagtt attagtccca ttttacagaa gaggaaactg agacttaggg 120
agatcaagta atttgcccag gtcgcacaat tagtgataga gccagggctt gaagcgacgt 180
ctgtcttaag ccaatgaccc ctgcagatta ttagagcaac tgttctccac aacagtgtaa 240
gcctcttgct anaagctcag gtccacaagg gcagagattt ttgtctgttt tgctcattgc 300
tccttcccca ttgcttagag cagggtctgc cacgaancag gttctcaatg catagttatt 360
aaatgtatat aagagcaaac atatgttaca gagaactttc tgtatgcttg tcacttacat 420
gaatcacctg tganatgggt atgcttgttc cccantgttg cagatnaaga tattgaangt 480
gcccaaatca ctanttgcgg gcgcctgcan gtccancata t 521

<210> 246
<211> 482
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(482)
<223> n = A,T,C or G

<400> 246
tggaaccaat ccaaataccc atcaatgata gactggataa agaaaatttg gcacatgttc 60
accatgaaat actatgcagc cataaaaaag gatgagttca tatcctttgc agggacatgg 120
atgaagctgg agaccatcat tctcagcaaa ctaacaaggg aacagaaaac caaacactgc 180
atgttctcac tcttaagtgg gagctgaaca atgagaacac atggacacag ggaggggaac 240
atcacacagt ggggcctgct ggtgggtagg ggtctagggg agggatagca ttaggagaaa 300
tacctaatgt agatgacggg ttgatgggtg cagcaaacca ccatgacacg tgtataccta 360
tgtaacaaac ctgcatgttc tgcacatgta ccccagaact taaagtgtta ataaaaaaat 420
taagaaaaaa gttaagtatg tcatagatac ataaaatatt gtanatattg aaggtgccca 480
aa 482

<210> 247
<211> 474

<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(474)
<223> n = A,T,C or G

<400> 247
ttcgatacag gcacagagta agcagaaaaa tggctgtggt ttaaccaagt gagtacagtt 60
aagtgagaga ggggcagaga agacaagggc atatgcaggg ggtgattata acaggtggtt 120
gtgctgggaa gtgagggtac tcggggatga ggaacagtga aaaagtggca aaaagtggta 180
agatcagtga attgtacttc tccagaattt gatttctggn ggagtcaaat aactatccag 240
tttggggtat catanggcaa cagttgaggt ataggaggta gaagtcncag tgggataatt 300
gaggttatga anggtttggt actgactggt actgacaang tctgggttat gaccatggga 360
atgaatgact gtanaagcgt anaggatgaa actattccac ganaaagggg tccnaaaact 420
aaaaannnaa gnnnnnggggg aatattattt atgtggatat tgaangtgcc caaa 474

<210> 248
<211> 355
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(355)
<223> n = A,T,C or G

<400> 248
ttcgatacag gcaaacatga actgcaggag ggtggtgacg atcatgatgt tgccgatggt 60
ccggatggnc acgaagacgc actggancac gtgcttacgt ccttttgctc tgttgatggc 120
cctgagggga cgcaggaccc ttatgaccct cagaatcttc acaacgggag atggcactgg 180
attgantccc antgacacca gagacacccc aaccaccagn atatcantat attgatgtag 240
ttcctgtaga nggccccctt gtggaggaaa gctccatnag ttggtcatct tcaacaggat 300
ctcaacagtt tccgatggct gtgatgggca tagtcatant taaccntgtn tcgaa 355

<210> 249
<211> 434
<212> DNA
<213> Homo sapien

<400> 249
ttggattggt cctccaggag aacaagggga aaaaggtgac cgagggctcc ctggaactca 60
aggatctcca ggagcaaaag gggatggggg aattcctggt cctgctggtc ccttaggtcc 120
acctggtcct ccaggcttac caggtcctca aggcccaaag ggtaacaaag gctctactgg 180
acccgctggc cagaaaggtg acagtggtct tccagggcct cctgggcctc caggtccacc 240
tggtgaagtc attcagcctt taccaatctt gtcctccaaa aaaacgagaa gacatactga 300
aggcatgcaa gcagatgcag atgataatat tcttgattac tcggatggaa tggaagaaat 360
atttggttcc ctcaattccc tgaaacaaga catcgagcat atgaaatttc caatgggtac 420
tcagaccaat ccaa 434

<210> 250
<211> 430
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(430)
<223> n = A,T,C or G

<400> 250
tggattggtc acatggcaga gacaggattc caaggcagtg agaggaggat acaatgcttc 60
tcactagtta ttattattta ttttatttt gagatgaagt ctcgctttgt ctcccaggct 120
ggagagcggt ggtgcgatct tggctctctg caacccccgc ctcaagcaat tctcctgtct 180
tagcctcgcg ggtagatgga attacaggcg cccaccgcca tgcccaacta attttttgt 240
gtcttcagta gagacagggt ttcgccatgt tgggcaggct ggtcttgaac tcctgacctc 300
nagtgatctg ccctcctcgg cctcacaaag tgctggaatt acaggcatgg gctgctgcac 360
ccagtcaact tctcactagt tatggcctta tcattttcac cacattctat tggcccaaaa 420
aaaaaaaaan 430

<210> 251
<211> 329
<212> DNA
<213> Homo sapien

<400> 251
tggtactcca ccatyatggg gtcaaccgcc atcctcgccc tcctcctggc tgttctccaa 60
ggagtctgtg ccgaggtgca gctgrtgcag tctggagcag aggtgaaaaa gtccggggag 120
tctctgaaga tctcctgtaa gggttctgga tacaccttta agatctactg gatcgcctgg 180
gtgcgccagt tgcccgggaa aggcctggag tggatggggc tcatctttcc tgatgactct 240
gataccagat acagcccgtc cttccaaggc caggtcacca tctcagtcga taagtccatc 300
agcaccgcct atctgcagtg gagtaccaa 329

<210> 252
<211> 536
<212> DNA
<213> Homo sapien

<400> 252
tggtactcca ctcagcccaa ccttaattaa gaattaagag ggaacctatt actattctcc 60
caggctcctc tgctctaacc aggcttctgg gacagtatta gaaaaggatg tctcaacaag 120
tatgtagatc ctgtactggc ctaagaagtt aaactgagaa tagcataaat cagaccaaac 180
ttaatggtcg ttgagacttg tgtcctggag cagctgggat aggaaaactt ttgggcagca 240
agaggaagaa ctgcctggaa gggggcatca tgttaaaaat tacaagggga acccacacca 300
ggccccttc ccagctctca gcctagagta ttagcatttc tcagctagag actcacaact 360
tccttgctta gaatgtgcca ccgggggggag tccctgtggg tgatgaggct ctcaagagtg 420
agagtggcat cctatcttct gtgtgcccac aggagcctgg cccgagactt agcaggtgaa 480
gtttctggtc caggctttgc ccttgactca ctatgtgacc tctggtggag taccaa 536

<210> 253
<211> 507

<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(507)
<223> n = A,T,C or G

<400> 253
ntgttgcgat cccagtaact cgggaagctg aggcgggagg atcacctgag ctcaggaggt 60
tgaggccgca gtgagccggg accacgccac tacactccag cctggggcat agagtgagac 120
cctccaagac agaaaagaaa agaaaggaag ggaaagggaa agggaaaagg aaaaggaaaa 180
ggaaaaggaa aaggaaaaga caagacaaaa caagacttga atttggatct cctgacttca 240
attttatgtt ctttctacac cacaattcct ctgcttacta agatgataat ttagaaaccc 300
ctcgttccat tctttacagc aagctggaag tttggtcaag taattacaat aatagtaaca 360
aatttgaata ttatatgcca ggtgtttttc attcctgctc tcacttaatt ctcaccactc 420
tgatataaat acaattgctg ccgggtgtgg tggctcatgc ctgtaatccc ggcactttgg 480
gagaccgagg tgggcggats gcaacaa 507

<210> 254
<211> 222
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(222)
<223> n = A,T,C or G

<400> 254
ttggattggt cactgtgagg aagccaaatc ggatccgaga gtctttttct aaaggccagt 60
actggccaca ctttctcctg ccgccttcct caaagctgaa gacacacaga gcaaggcgct 120
tctgttttac tccccaatgg taactccaaa ccatagatgg ttagctnccc tgctcatctt 180
tccacatccc tgctattcag tatagtccgt ggaccaatcc aa 222

<210> 255
<211> 463
<212> DNA
<213> Homo sapien

<400> 255
tgttgcgatc cataaatgct gaaatggaaa taaacaacat gatgagggag gattaagttg 60
gggagggagc acattaaggt ggccatgaag tttgttggaa gaagtgactt ttgaacaagg 120
ccttggtgtt aagagctgat gagagtgtcc cagacagagg ggccactggt acaatagacg 180
agatgggaga gggcttggaa ggtgtgcgaa ataggaagga gtttgttctg gtatgagtct 240
agtgaacaca gaggcgagag gccctggtgg gtgcagctgg agagttatgc agaataacat 300
taggccctgt gggggactgt agactgtcag caataatcca cagtttggat tttattctaa 360
gagtgatggg aagccgtgga aaggggggtta agcaaggagt gaaattatca gatttacagt 420
gataaaaata aattggtctg gctactgggg aaaaaaaaaa aaa 463

<210> 256

<211> 262
<212> DNA
<213> Homo sapien

<400> 256
ttggattggt caacctgctc aactctacyt ttcctccttc ttcctaaaaa attaatgaat 60
ccaatacatt aatgccaaaa cccttgggtt ttatcaatat ttctgttaaa aagtattatc 120
cagaactgga cataatacta cataataata cataacaacc ccttcatctg gatgcaaaca 180
tctattaata tagcttaaga tcactttcac tttacagaag caacatcctg ttgatgttat 240
tttgatgttt ggaccaatcc aa 262

<210> 257
<211> 461
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(461)
<223> n = A,T,C or G

<400> 257
gnggnnnnnn nnncaattcg actcngttcc cntggtancc ggtcgacatg gccgcgggat 60
taccgcttgt nnctgggggt gtatggggga ctatgaccgc ttgtagctgg gggtgtatgg 120
gggactatga ccgcttgtag mtggkggtgt atgggggact atgaccgctt gtcgggtggt 180
cggataaacc gacgcaaggg acgtgatcga agctgcgttc ccgctctttc gcatcggtag 240
ggatcatgga cagcaatatc cgcattcgyc tgaaggcgtt cgaccatcgc gtgctcgatc 300
aggcgaccgg cgacatcgcc gacaccgcac gccgtaccgg cgcgctcatc cgcggtccga 360
tcccgcttcc cacgcgcatc gagaagttca cggtcaaccg tggcccgcac gtcgacaaga 420
agtcgcgcga gcagttcgag gtgcgtacct acaagcggtc a 461

<210> 258
<211> 332
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(332)
<223> n = A,T,C or G

<400> 258
tgaccgcttg tagctggggg tgtatggggg actacgaccg cttgtagctg ggggtgtatg 60
ggggactatg accgcttgta gctgggggtg tatgggggac tatgaccgct tgtagctggg 120
ggtgtatggg ggactaggac cgcttgtagc tgggggtgta tgggggacta tgaccgcttg 180
tagctggggg tgtatggggg actacgaccg cttgtagctg ggggtgtatg ggggactatg 240
accgcttgta nctgggggtg tatgggggac tatgaccgct tgtgctgcct gggggatggg 300
aggagagttg tggttgggga aaaaaaaaaa aa 332

<210> 259
<211> 291

<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(291)
<223> n = A,T,C or G

<400> 259
taccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt 60
gaccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt 120
gaccgcttgt gaccgcttgt nacngggggt gtctggggga ctatgannga ntgtnactgg 180
gggtgtctgg gggnctatga nngantgtna cnggggggtgt ctgggggact atganngact 240
gtgcnncctg ggggatcnga ggagantngn ggntagngat ggttngggan a 291

<210> 260
<211> 238
<212> DNA
<213> Homo sapien

<400> 260
taagagggta ctggttaaaa tacaggaaat ctggggtaat gaggcagaga accaggatac 60
tttgaggtca gggatgaaaa ctagaatttt tttctttttt tttgcctgag aaacttgctg 120
ctctgaagag gcccatgtat taattgcttt gatcttcctt ttcttacagc cctttcaagg 180
gcagagccct ccttatcctg aaggaatctt atccttagct atagtatgta ccctctta 238

<210> 261
<211> 746
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(746)
<223> n = A,T,C or G

<400> 261
ttgggcacct tcaatatcaa tagctaacat ttattgagtg tttatcgtat cataaaacac 60
tgttctaagc ctttaaacgt actaattcat ttaatgctca taatcacttt agaaggtggg 120
tactagtatt agtctcattt acagatgcaa catgcaggca cagagaggtt aattaacttg 180
cccaaggtaa cacagctaag aaatagaaaa aatattgaat ctggaaagtt gggcttctgg 240
gtaacccaca gagtcttcaa tgagcctggg gcctcactca gtttgctttt acaaagcgaa 300
tgagtaacat cacttaattc agtgagtagg ccaaatggag gtcagctacg agtttctgct 360
gttcttgcag tggactgaca gatgtttaca acgtctggcc atcagtwaat ggactgatta 420
tcattgggaw gtgggtgggc tgaatgttgg ccagtgaagt ttattcawgc catattttta 480
tgtttaggat gacttttggc tggtcctagg gcaagctctg tctgscacgg aacacagaat 540
wacacaggga ccccctcaat ttctggtgtg gctagaacca tgaaccactg gttgggggaa 600
caagcggtca aaacctaagt gcggccggct ggcagggtcc acccatatgg ggaaaactcc 660
cnacgcgttt ggaatgcctn agctngaatt attctaanag ttgtccncnt aaaattagcc 720
tgggcgttaa tcangggtcn naagcc 746

<210> 262
<211> 588
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(588)
<223> n = A,T,C or G

<400> 262
tgaccgcttg tcatctcaca tggggtcctg cacgcttttg cctttgtagg aaacctgaca 60
tttgtctgtt tcttctttct cttttccttc ccatatcctc ctaatttacg tttgacttgt 120
ttgctgagga ggcaggagct agagactgct gtgagctcat aggggtggga agtttatcct 180
tcaagtcccg cccactcatc actgcttctc accttcccct gaccaggctt acaagtgggt 240
tcttgcctgc tttccctttg gacccaacaa gcccctgtaa tgagtgtgca tgactctgac 300
agctgtggac tcagggtcct tggctacagc tgccatgtaa aatatctcat ccagttctcg 360
caaattgtta aaataaccac atttcttaga ttccagtacc caaatcatgt ctttacgaac 420
tgctcctcac acccagaagt ggcacaataa ttcttgggga attattactt ttttttttct 480
ctctnttnnc gnnngnnnng gnnngnccag gaattaccac nttggaagac ctggccngaa 540
tttattatan aggggagccg attnttttttc ctaacacaaa gcgggtca 588

<210> 263
<211> 730
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(730)
<223> n = A,T,C or G

<400> 263
ttttttttttt tttggcctga gcaactgaaa ttatgaaatt tccatatact caaaagagta 60
agactgcaaa aagattaaat gtaaaagttg tcttgtatac agtaatgttt aagataccta 120
ttanatttat aaatggaaaa ttagggcatt tggatataca agttgaaaat tcaggagtga 180
ggttgggctg gctgggtata tactgaaaac tgtcagtaca cagatgacat ctaaaaccac 240
aaatctggtt ttatttttagc agtgatatgt gtcactccca caaaagcctt cccaattggc 300
ctcagcatac acaacaagtc acctccccac agccctctac acataaacaa attccttagt 360
ttagttcagg aggaaatgcg ccctttttcct tccgctctag gtgaccgcaa ggcccagttc 420
tcgtcaccaa gatgttaagg gaagtctgcc aaagaggcat ctgaaaggaa ataaggggaa 480
tgggagtgac cacaaaggaa agccaaggan aaactttgga gaccgtttct agancctgg 540
catttcacaa caaaactcng gaacaaacct tgtctcatca atcatttaag cccttcgttt 600
ggannagact ttctgaactg ggcgctgaac ataancctca ttgaatgtct tcacagtctc 660
ccagctgaag gcacaccttg ggccagaagg ggaatcttcc aggtcctcaa nacagggctc 720
gcccttgnc 730

<210> 264
<211> 715
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(715)
<223> n = A,T,C or G

<400> 264
ttttttttt tttggccagt atgatagtct ctaccactat attgaagctc ttaggtcatt 60
tacacttaat gtggttatag atgctgttga gcttacttct accaccttgc tatttctccc 120
gtctcttttt tgttcctttt ctcttctttt cctcccttat tttataattg aatttttag 180
gattctattt tatatagatt tatcagctat aacactttgt attcttttgt tttgtggttc 240
ttctgtcatt tcaatgtgca tcttaaactc atcacaatct attttcaaat aatatcatat 300
aaccttacat ataatgtaag aatctaccac catatatttc catttctccc ttccatccta 360
tgtntgtcat attttttcct ttatatatgt tttaaagaca taatagtata tgggaggttt 420
ttgcttaaaa tgtgatcaat attccttcaa ngaaacgtaa aaattcaaaa taaatntctg 480
tttattctca aatnnaccta atatttccta ccatntctna tacntttcaa gaatctgaag 540
gcattggttt tttccggctt aagaacctcc tctaaagcac tctaagcaga attaagtctt 600
ctgggagagg aattctccca agcttgggcc ttnanntgta ctccntnang gttaaanttt 660
ggccgggaaa tagaaattcc aagttaacag gntantttt ntttttnttn tcncc 715

<210> 265
<211> 152
<212> DNA
<213> Homo sapien

<400> 265
ttttttttt tttcccaaca caaagcacca ttatctttcc tcacaatttt caacatagtt 60
tgattcccat gaagaggtta tgatttctaa agaaaacatg gctactatac tatcaatcag 120
ggttaaatct ttttttttg agacggagtt ta 152

<210> 266
<211> 193
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(193)
<223> n = A,T,C or G

<400> 266
taaactccgt ccccttctta atcaatatgg aggctaccca ctccacatta ccttcttttc 60
aagggactgt ttccgtaact gttgtgggta ttcacgacca ggcttctaaa cctcttaaaa 120
ctccccaatt ctggtgccaa cttggacaac atgcttttt ttttttttt ttttttttn 180
gagacggagt tta 193

<210> 267
<211> 460
<212> DNA
<213> Homo sapien

<400> 267
tgttgcgatc ccttaagcat gggtgctatt aaaaaaatgg tggagaagaa aatacctgga 60
atttacgtct tatctttaga gattgggaag accctgatgg aggacgtgga gaacagcttc 120
ttcttgaatg tcaattccca agtaacaaca gtgtgtcagg cacttgctaa ggatcctaaa 180
ttgcagcaag gctacaatgc tatgggattc tcccagggag gccaatttct gagggcagtg 240
gctcagagat gcccttcacc tcccatgatc aatctgatct cggttggggg acaacatcaa 300
ggtgtttttg gactccctcg atgcccagga gagagctctc acatctgtga cttcatccga 360
aaaacactga atgctggggc gtactccaaa gttgttcagg aacgcctcgt gcaagccgaa 420
tactggcatg acccataaaa ggaggatgtg gatcgcaaca 460

<210> 268
<211> 533
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(533)
<223> n = A,T,C or G

<400> 268
tgttgcgatc cgttgataga atagcgacgt ggtaatgagt gcatggcacg cctccgactt 60
accttcgccc gtggggaccc cgagtacgtc tacggcgtcg tcacttagag taccctctgg 120
acgcccgggc gcgttcgatt taccggaagc gcgagctgca gtgggcttgc gccccggcc 180
aaattctttg gggggtttaa ggccgcgggg aatttgaggt atctctatca gtatgtagcc 240
aagttggaac agtcgccatt cccgaaatcg ctttctttga atccgcaccg cctccagcat 300
tgcctcattc atcaacctga aggcacgcat aagtgacggt tgtgtcttca gcagctccac 360
tccataacta gcgcgctcga cctcgtcttc gtacgcgcca ggtccgtgcg tgcgaattcc 420
caactccggt gagttgcgca tttcaagttn cgaaactgtt cgcctccacn atttggcatg 480
ttcacgcatg acacggaata aactcgtcca gtaccgggaa tgggatcgca aca 533

<210> 269
<211> 50
<212> DNA
<213> Homo sapien

<400> 269
ttttttttt ttcgcctgaa ttagctacag atcctcctca caagcggtca 50

<210> 270
<211> 519
<212> DNA
<213> Homo sapien

<400> 270
tgttgcgatc caaataaccc accagcttct tgcacacttc gcagaagcca ccgtcctttg 60
gctgagtcac gtgaacggtc agtgcaagca gccgcgtgcc agagcagagg tgcagcatgc 120
tgcacaccag ctcagggctg acctcctcca gcaggatgga caggatggag ctgccgtacg 180
tgtccaccac ctcctggcac tcttccgaca gggacttcgg cagcttcgag cacattttgt 240
caaaagcgtc gagtatttct ttctcagtct tgttgttgtc aatcagcttg gtcacctcct 300
tcaccaggaa ttcacacacc tcacagtaaa catcagactt tgctgggacc tcgtgcttct 360

taatgggctc caccagttcc agggcaggga tgacattctt ggaggccact ttggcgggga 420
ccagagtctg catgggcatc tctttcacct catcacagaa cccaaccagc gcacagatct 480
ccttgggttg catgtgcatc atcatctggg atcgcaaca 519

<210> 271
<211> 457
<212> DNA
<213> Homo sapien

<400> 271
ttttttttt ttcgggcggc gaccggacgt gcactcctcc agtagcggct gcacgtcgtg 60
ccaatggccc gctatgagga ggtgagcgtg tccggcttcg aggagttcca ccgggccgtg 120
gaacagcaca atggcaagac cattttcgcc tactttacgg gttctaagga cgccgggggg 180
aaaagctggt gccccgactg cgtgcaggct gaaccagtcg tacgagaggg gctgaagcac 240
attagtgaag gatgtgtgtt catctactgc caagtaggag aagagcctta ttggaaagat 300
ccaaataatg acttcagaaa aaacttgaaa gtaacagcag tgcctacact acttaagtat 360
ggaacacctc aaaaactggt agaatctgag tgtcttcagg ccaacctggt ggaaatgttg 420
ttctctgaag attaagattt taggatggca atcaaga 457

<210> 272
<211> 102
<212> DNA
<213> Homo sapien

<400> 272
ttttttttt ttgggcaaca acctgaatac cttttcaagg ctctggcttg ggctcaagcc 60
cgcaggggaa atgcaactgg ccaggtcaca gggcaatcaa ga 102

<210> 273
<211> 455
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(455)
<223> n = A,T,C or G

<400> 273
ttttttttt ttggcaatca acaggtttaa gtcttcggcc gaagttaatc tcgtgttttt 60
ggcaatcaac aggtttaagt cttcggccga agttaatctc gtgtttttgg caatcaacag 120
gtttaagtct tcggccgaag ttaatctcgt gtttttggca atcaacaggt ttaagtcttc 180
ggccgaagtt aatctcgtgt ttttggcaat caacaggttt aagtcttcgg ccgaagttaa 240
tctcgtgttt ttggcaatca acaggtttaa gtcttcggcc gaagttaatc tcgtgttttt 300
ggcaatcaag aggtttaagt cttcggccga agttaatctc gtgtttttgg caatcaacag 360
gtttaagtct tcggccgaan ttaatctcgt gtttttggca atcaacaggt ttaantcttc 420
ggccgaagtt aatctcgtgt ttttggcaat caana 455

<210> 274
<211> 461
<212> DNA

<213> Homo sapien

<400> 274
ttttttttt ttggccaata cccttgatga acatcaatgt gaaaatcctc ggtaaaatac 60
tggcaaacca aatccagcag cacatcaaaa agcttatcca ccatgatcaa gtgggcttca 120
tccctgggat gcaaggctgg ttcaacataa gaaaatcaat aaatgtaatc catcacataa 180
acagaaccaa agacaaaaac cacatgatta tctcaataga tgcagaaaag gccttggaca 240
aattcaacag cccttcatgc taaacactct taataaacta gatattgatg gaatgtatct 300
caaaataata agagctattt atgacaaacc cacagccaat atcatactga atgggcaaag 360
actggaagca ttccctttga aaactggcac aagacaagga tgccctctct caccgctcct 420
attcaacata gtattggaag ttctggccag ggcaatcaag a 461

<210> 275
<211> 729
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(729)
<223> n = A,T,C or G

<400> 275
ttttttttt ttggccaaca ccaagtcttc cacgtgggag gttttattat gttttacaac 60
catgaaaaca taggaaggtg gctgttacag caaacatttc agatagacga atcggccaag 120
ctccccaaac cccaccttca cagcctcttc cacacgtctc ccanagattg ttgtccttca 180
cttgcaaatt canggatgtt ggaagtngac atttnnagtn gcnggaaccc catcagtgaa 240
ncantaagca gaantacgat gactttgana nacanctgat gaagaacacn ctacnganaa 300
ccctttctnt cgtgttanga tctcnngtcc ntcactaatg cggccccctg cnggtccacc 360
atttgggaga actccccccn cgttggatcc ccccttgagt ntcccattct ngtcccccan 420
accngncttg ngngncantn cnncctcnca ccntgtttcc ctgnngtnaa aatnngtttt 480
nccgccnccc naattcccac ccnaatcaca gcgaanccng aaggccttcn naagtgttta 540
angcccngng gtttcctcnt ntanttgcag cctaccctcc cncttnnnnt tncgngttgg 600
tcgcgccctg gncncgcctn gttcctcttt nnggnnacaa cctngntcnn nggcncntcn 660
nnnctnttcc tnnnactagc tngcctntcc ncnccgnggn ncanngcaca ttncncnnac 720
tntgtnncc 729

<210> 276
<211> 339
<212> DNA
<213> Homo sapien

<400> 276
tgacctgaca tgtagtagat acttaataaa tatttgtgga atgaatggat gaagtggagt 60
tacagagaaa aatagaaaag tacaaattgt tgtcagtgtt ttgaaggaaa attatgatct 120
ttcccaaagt tctgacttca ttctaagaca gggttagtat ctccatacat aattttactt 180
gcttttgaaa atcaaatgag ataatctatt tagattgata atttatttag actggctata 240
aactattaag tgctagcaaa tatacatttt aatctcattt tccacctctt gtgatatagc 300
tatgtaggtg ttgactttaa tggatgtcag gtcaatccc 339

<210> 277

<211> 664
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(664)
<223> n = A,T,C or G

<400> 277
tgacctgaca tccataacaa aatctttctc cattatattc ttctagggga atttcttgaa 60
aagcatccaa aggaaacaaa tgatggtaag accgtgccaa gtggggagca gacaccaaag 120
taagaccaca gattttacat tcaacaggta gctcacagta ctttgcccga cactgtgggc 180
agaaatagcc tcctaatgta agccctggct cagtattgcc atccaaatgc gccatgctga 240
aagagggttt tgcatcctgg tcagatnaag aagcaatggt gtgctgagga aatcccatac 300
gaataagtga gcattcagaa cttgagctag caggaggagg actaagatga tgtgtgagca 360
actctttgta atggctttca tctaaaataa catggtacgt gccaccagtt tcacgagcaa 420
gtacagtgca aacgcgaact tctgcagaca atccaataac agatactcta attttagctg 480
cctttagggt cttgattaaa tcataaatat tagatggatc gcaagttgta aggntgctaa 540
aagatgatta gtacttctcg acttgtatgt ccaggcatgt tgttttaaan tctgccttag 600
nccctgctta ggggaatttt taaagaagat ggctctccat gttcanggtc aatcacnaat 660
tgcc 664

<210> 278
<211> 452
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(452)
<223> n = A,T,C or G

<400> 278
tgacctgaca ttgaggaaga gcacacacct ctgaaattcc ttaggttcag aagggcattt 60
gacacagagt gggcctctga taattcatga aatgcattct gaagtcatcc agaatggagg 120
ctgcaatctg ctgtgctttg ggggttgcct cactgtgctc ctggatatca cacaaaagct 180
gcaatccttc ttcttcaact aacattttgc agtatttgct gggattttta ctgcagacat 240
gatacatagc ccatagtgcc cagagctgaa cctctggttg agagaagttg ccaaggagcg 300
ggaaaaatgt cttgaaagat ctataggtca ccaatgctgt catcttacaa cttgaacttg 360
gccaattctg tatggttgca tgcagatctt ggagaagagt acgcctctgg aagtcacggg 420
atatccaaan ctgtctgtca gatgtcaggt ca 452

<210> 279
<211> 274
<212> DNA
<213> Homo sapien

<400> 279
ttttttttttt ttcggcaagg caaatttact tctgcaaaag ggtgctgctt gcacttttgg 60
ccactgcgag agcacaccaa acaaagtagg gaaggggttt ttatccctaa cgcggttatt 120

ccctggttct gtgtcgtgtc cccattggct ggagtcagac tgcacaatct acactgaccc 180
aactggctac tgtttaaaat tgaatatgaa taattaggta ggaaggggga ggctgtttgt 240
tacggtacaa gacgtgtttg ggcatgtcag gtca 274

<210> 280
<211> 272
<212> DNA
<213> Homo sapien

<400> 280
tacctgacat ggagaaataa cttgtagtat tttgcgtgca atggaatact atatgagggt 60
gaaaatgaat gaactagcaa tgcgtgtatc aacatgaata aatccccaaa acataataat 120
gttgaatgga aaaggtgagt ttcagaagga tatatatgcc ctctaaatcc atttatgtaa 180
acctttaaaa aactacatta tttatggtca taagtccatc cagaaaatat ttaaaaacct 240
acatgggatt gataactact gatgtcaggt ca 272

<210> 281
<211> 431
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(431)
<223> n = A,T,C or G

<400> 281
ttttttttttt ttggccaata gcatgattta aacattggaa aaagtcaaat gagcaatgcg 60
aatttttatg ttctcttgaa taatcaaaag agtaggcaac attggttcct cattcttgaa 120
tagcattaat cagaaaatat tgcatagcct ctagcctcct tagagtaggt gtgctctctc 180
aaatatatca tagtcccaca gtttatttca tgtatatttt ctgcctgaat cacatagaca 240
tttgaatttg caacgcctga tgtaaatata taaattctta ccaatcagaa acatagcaag 300
aaattcaggg acttggtcat yatcagggta tgacagcana tccctgtara aacactgata 360
cacactcaca cacgtatgca acgtggagat gtcgcyttww kkktwywcwm rmrycrwcgn 420
aatcacttan n 431

<210> 282
<211> 98
<212> DNA
<213> Homo sapien

<400> 282
attcgattcg atgcttgagc ccaggagttc aagactgcag tgagccactg cacttcaggc 60
tggacaacag agcgagtccc tgtgccaaaa aaaaaaaa 98

<210> 283
<211> 764
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(764)
<223> n = A,T,C or G

<400> 283
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cgtttttcct gtattatctg taacataata tggtagactg tcacagagcc gaatwccart 240
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ngnnanttct ttccttccct cccnacgcnn tgcgtgcgcc cgtctngcct nnnctncgna 660
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<210> 284
<211> 157
<212> DNA
<213> Homo sapien

<400> 284
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atttctcccc ttccaggaac gtcttgcatg gatgatcaaa gatcagctcc tggtcaacat 120
aaataagcta gtttaagata cgttccccta cacttga 157

<210> 285
<211> 150
<212> DNA
<213> Homo sapien

<400> 285
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tagatgagca gctgcctagg tctgagtaca 150

<210> 286
<211> 219
<212> DNA
<213> Homo sapien

<400> 286
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gcaaccttgg ttaggatcaa tccaatattc accatctggg aagtcaggat ggctgagttg 180
caggtcttta caagttcggg ctggattggt ctgagtaca 219

<210> 287

<211> 196
<212> DNA
<213> Homo sapien

<400> 287
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actgtgagag agtacatttc tcttggttta agccaagaga atctgtcttt tggtacttta 180
tatcatagcc tcaaga 196

<210> 288
<211> 199
<212> DNA
<213> Homo sapien

<400> 288
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taggtactga agattcaagt gaccgagatg ctagcccttg ggttcaagtg atccctctcc 180
cagagtgcac tggactgaa 199

<210> 289
<211> 182
<212> DNA
<213> Homo sapien

<400> 289
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gtattataat cttagggacc accattatat atgtggtcca tcattggcca aaaaaaaaaa 180
aa 182

<210> 290
<211> 1646
<212> DNA
<213> Homo sapien

<400> 290
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      <210> 291
      <211> 1851
      <212> DNA
      <213> Homo sapien

      <400> 291
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gctcctgaga aacaccccag ctcttccggt ctaacacagg caagtcaata aatgtgataa    1620
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<210> 292
<211> 1851
<212> DNA
<213> Homo sapien

<400> 292

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tcacttcctt taagcctttg tgactcttcc tctgatgtca gctttaagtc ttgttctgga    180
ttgctgtttt cagaagagat ttttaacatc tgtttttctt tgtagtcaga aagtaactgg    240
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aaggtatgtc ccttctatgc ctgttttgct gagggtttta attctcgtgc c             1851
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<210> 293
<211> 668
<212> DNA
<213> Homo sapien

<400> 293

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accrtataag agcagtgctt tggccattaa tttatctttc attrtagaca gcrtagtgya    180
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gcagtcctat gagagtgaga agacttttta ggaaattgta gtgcactagc tacagccata 600
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aaaaaaaa 668

<210> 294
<211> 1512
<212> DNA
<213> Homo sapien

<400> 294
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<210> 295
<211> 1853
<212> DNA
<213> Homo sapien

<400> 295
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<211> 2184
<212> DNA
<213> Homo sapien

<400> 296
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agacttaaag ctgacatcag aggaagagtc acaaaggctt aaaggaagtg aaaacagcca 1740
gccagaggca tggaaacttt taaatttaaa cttttggttt aatgtttttt ttttttgcct 1800
taataatatt agatagtccc aaatgaaatw acctatgaga ctaggctttg agaatcaata 1860
gattcttttt ttaagaatct tttggctagg agcggtgtct cacgcctgta attccagcac 1920
cttgagaggc tgaggtgggc agatcacgag atcaggagat cgagaccatc ctggctaaca 1980
cggtgaaacc ccatctctac taaaaatata aaaacttagc tgggtgtggt ggcgggtgcc 2040
tgtagtccca gctactcagg argctgaggc aggagaatgg catgaacccg ggaggtggag 2100
gttgcagtga gccgagatcc gccactacac tccagcctgg gtgacagagc aagactctgt 2160
ctcaaaaaaa aaaaaaaaaa aaaa 2184

<210> 297
<211> 1855
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(1855)
<223> n = A,T,C or G

<400> 297
tgcacgcatc ggccagtgtc tgtgccacgt acactgacgc cccctgagat gtgcacgccg 60
cacgcgcacg ttgcacgcgc ggcagcggct tggctggctt gtaacggctt gcacgcgcac 120
gccgcccccg cataaccgtc agactggcct gtaacggctt gcaggcgcac gccgcacgcg 180
cgtaacggct tggctgccct gtaacggctt gcacgtgcat gctgcacgcg cgttaacggc 240
ttggctggca tgtagccgct tggcttggct ttgcattytt tgctkggctk ggcgttgkty 300
tcttggattg acgcttcctc cttggatkga cgtttcctcc ttggatkgac gtttcytyty 360
tcgcgttcct ttgctggact tgacctttty tctgctgggt ttggcattcc tttggggtgg 420
gctgggtgtt ttctccgggg gggktkgccc ttcctggggt gggcgtgggk cgcccccagg 480
gggcgtgggc tttccccggg tgggtgtggg ttttcctggg gtggggtggg ctgtgctggg 540
atccccctgc tggggttggc agggattgac ttttttcttc aaacagattg gaaacccgga 600
gtaacntgct agttggtgaa actggttggt agacgcgatc tgctggtact actgtttctc 660
ctggctgtta aaagcagatg gtggctgagg ttgattcaat gccggctgct tcttctgtga 720
agaagccatt tggtctcagg agcaagatgg gcaagtggtg cgccactgct tcccctgctg 780
caggggagc ggcaagagca acgtgggcac ttctggagac cacaacgact cctctgtgaa 840
gacgcttggg agcaagaggt gcaagtggtg ctgcccactg cttcccctgc tgcaggggag 900
cggcaagagc aacgtggkcg cttggggaga ctacgatgac agcgccttca tggakcccag 960
gtaccacgtc crtggagaag atctggacaa gctccacaga gctgcctggt ggggtaaagt 1020
ccccagaaag gatctcatcg tcatgctcag ggacactgay gtgaacaaga rggacaagca 1080

```
aaagaggact gctctacatc tggcctctgc caatgggaat tcagaagtag taaaactcgt    1140
gctggacaga cgatgtcaac ttaatgtcct tgacaacaaa aagaggacag ctctgacaaa    1200
ggccgtacaa tgccaggaag atgaatgtgc gttaatgttg ctggaacatg gcactgatcc    1260
aaatattcca gatgagtatg gaaataccac tctacactat gctgtctaca atgaagataa    1320
attaatggcc aaagcactgc tcttatacgg tgctgatatc gaatcaaaaa acaaggtata    1380
gatctactaa ttttatcttc aaaatactga aatgcattca ttttaacatt gacgtgtgta    1440
agggccagtc ttccgtattt ggaagctcaa gcataacttg aatgaaaata ttttgaaatg    1500
acctaattat ctaagacttt attttaaata ttgttatttt caaagaagca ttagagggta    1560
cagttttttt tttttaaatg cacttctggt aaatactttt gttgaaaaca ctgaatttgt    1620
aaaaggtaat acttactatt tttcaatttt tccctcctag gatttttttc ccctaatgaa    1680
tgtaagatgg caaaatttgc cctgaaatag gttttacatg aaaactccaa gaaaagttaa    1740
acatgtttca gtgaatagag atcctgctcc tttggcaagt tcctaaaaaa cagtaataga    1800
tacgaggtga tgcgcctgtc agtggcaagg tttaagatat ttctgatctc gtgcc          1855
```

<210> 298
<211> 1059
<212> DNA
<213> Homo sapien

<400> 298

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gcaacgtggg cacttctgga gaccacaacg actcctctgt gaagacgctt gggagcaaga      60
ggtgcaagtg gtgctgccca ctgcttcccc tgctgcaggg gagcggcaag agcaacgtgg     120
gcgcttgrgg agactmcgat gacagygcct tcatggagcc caggtaccac gtccgtggag     180
aagatctgga caagctccac agagctgccc tggtggggta aagtccccag aaaggatctc     240
atcgtcatgc tcagggacac tgaygtgaac aagarggaca agcaaaagag gactgctcta     300
catctggcct ctgccaatgg gaattcagaa gtagtaaaac tcstgctgga cagacgatgt     360
caacttaatg tccttgacaa caaaaagagg acagctctga yaaaggccgt acaatgccag     420
gaagatgaat gtgcgttaat gttgctggaa catggcactg atccaaatat tccagatgag     480
tatggaaata ccactctrca ctaygctrtc tayaatgaag ataaattaat ggccaaagca     540
ctgctcttat ayggtgctga tatcgaatca aaaaacaagg tatagatcta ctaattttat     600
cttcaaaata ctgaaatgca ttcattttaa cattgacgtg tgtaagggcc agtcttccgt     660
atttggaagc tcaagcataa cttgaatgaa aatattttga aatgacctaa ttatctaaga     720
ctttatttta aatattgtta ttttcaaaga agcattagag ggtacagttt ttttttttta     780
aatgcacttc tggtaaatac ttttgttgaa aacactgaat ttgtaaaagg taatacttac     840
tatttttcaa tttttccctc ctaggatttt tttcccctaa tgaatgtaag atggcaaaat     900
ttgccctgaa ataggtttta catgaaaact ccaagaaaag ttaaacatgt ttcagtgaat     960
agagatcctg ctccttggc aagttcctaa aaaacagtaa tagatacgag gtgatgcgcc    1020
tgtcagtggc aaggtttaag atatttctga tctcgtgcc                          1059
```

<210> 299
<211> 329
<212> PRT
<213> Homo sapien

<400> 299

```
Met Asp Ile Val Val Ser Gly Ser His Pro Leu Trp Val Asp Ser Phe
 1               5                  10                  15
Leu His Leu Ala Gly Ser Asp Leu Leu Ser Arg Ser Leu Met Ala Glu
            20                  25                  30
Glu Tyr Thr Ile Val His Ala Ser Phe Ile Ser Cys Ile Ser Ser Ser
        35                  40                  45
```

```
Leu Asp Gly Gln Gly Glu Arg Gln Glu Gln Arg Gly His Phe Trp Arg
    50                  55                  60
Pro Gln Arg Leu Leu Cys Glu Asp Ala Trp Glu Gln Glu Val Gln Val
65                  70                  75                  80
Val Leu Pro Leu Leu Pro Leu Leu Gln Gly Ser Gly Lys Ser Asn Val
                85                  90                  95
Val Ala Trp Gly Asp Tyr Asp Asp Ser Ala Phe Met Asp Pro Arg Tyr
            100                 105                 110
His Val His Gly Glu Asp Leu Asp Lys Leu His Arg Ala Ala Trp Trp
        115                 120                 125
Gly Lys Val Pro Arg Lys Asp Leu Ile Val Met Leu Arg Asp Thr Asp
    130                 135                 140
Val Asn Lys Arg Asp Lys Gln Lys Arg Thr Ala Leu His Leu Ala Ser
145                 150                 155                 160
Ala Asn Gly Asn Ser Glu Val Val Lys Leu Val Leu Asp Arg Arg Cys
                165                 170                 175
Gln Leu Asn Val Leu Asp Asn Lys Lys Arg Thr Ala Leu Thr Lys Ala
            180                 185                 190
Val Gln Cys Gln Glu Asp Glu Cys Ala Leu Met Leu Leu Glu His Gly
        195                 200                 205
Thr Asp Pro Asn Ile Pro Asp Glu Tyr Gly Asn Thr Thr Leu His Tyr
    210                 215                 220
Ala Val Tyr Asn Glu Asp Lys Leu Met Ala Lys Ala Leu Leu Leu Tyr
225                 230                 235                 240
Gly Ala Asp Ile Glu Ser Lys Asn Lys His Gly Leu Thr Pro Leu Leu
                245                 250                 255
Leu Gly Ile His Glu Gln Lys Gln Gln Val Val Lys Phe Leu Ile Lys
            260                 265                 270
Lys Lys Ala Asn Leu Asn Ala Leu Asp Arg Tyr Gly Arg Thr Ala Leu
        275                 280                 285
Ile Leu Ala Val Cys Cys Gly Ser Ala Ser Ile Val Ser Pro Leu Leu
    290                 295                 300
Glu Gln Asn Val Asp Val Ser Ser Gln Asp Leu Glu Arg Arg Pro Glu
305                 310                 315                 320
Ser Met Leu Phe Leu Val Ile Ile Met
                325
```

<210> 300
<211> 148
<212> PRT
<213> Homo sapien

<220>
<221> VARIANT
<222> (1)...(148)
<223> Xaa = Any Amino Acid

<400> 300

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Met Thr Xaa Pro Ser Trp Ser Pro Gly Thr Thr Ser Val Glu Lys Ile
 1               5                  10                  15
Trp Thr Ser Ser Thr Glu Leu Pro Trp Trp Gly Lys Val Pro Arg Lys
```

```
            20                  25                  30
Asp Leu Ile Val Met Leu Arg Asp Thr Asp Val Asn Lys Xaa Asp Lys
        35                  40                  45
Gln Lys Arg Thr Ala Leu His Leu Ala Ser Ala Asn Gly Asn Ser Glu
    50                  55                  60
Val Val Lys Leu Xaa Leu Asp Arg Arg Cys Gln Leu Asn Val Leu Asp
65                  70                  75                  80
Asn Lys Lys Arg Thr Ala Leu Xaa Lys Ala Val Gln Cys Gln Glu Asp
                85                  90                  95
Glu Cys Ala Leu Met Leu Leu Glu His Gly Thr Asp Pro Asn Ile Pro
            100                 105                 110
Asp Glu Tyr Gly Asn Thr Thr Leu His Tyr Ala Xaa Tyr Asn Glu Asp
        115                 120                 125
Lys Leu Met Ala Lys Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu Ser
    130                 135                 140
Lys Asn Lys Val
145
```

<210> 301
<211> 1155
<212> DNA
<213> Homo sapien

<400> 301

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aggagcaaga tgggcaagtg gtgctgccgt tgcttccccc gctgcaggga gagcggcaag     120
agcaacgtgg gcacttctgg agaccacgac gactctgcta tgaagacact caggagcaag     180
atgggcaagt ggtgccgcca ctgcttcccc tgctgcaggg ggagtggcaa gagcaacgtg     240
ggcgcttctg gagaccacga cgactctgct atgaagacac tcaggaacaa gatgggcaag     300
tggtgctgcc actgcttccc ctgctgcagg gggagcggca agagcaaggt gggcgcttgg     360
ggagactacg atgacagtgc cttcatggag cccaggtacc acgtccgtgg agaagatctg     420
gacaagctcc acagagctgc ctggtggggt aaagtcccca gaaaggatct catcgtcatg     480
ctcagggaca ctgacgtgaa caagaaggac aagcaaaaga ggactgctct acatctggcc     540
tctgccaatg ggaattcaga agtagtaaaa ctcctgctgg acagacgatg tcaacttaat     600
gtccttgaca acaaaaagag gacagctctg ataaaggccg tacaatgcca ggaagatgaa     660
tgtgcgttaa tgttgctgga acatggcact gatccaaata ttccagatga gtatggaaat     720
accactctgc actacgctat ctataatgaa gataaattaa tggccaaagc actgctctta     780
tatggtgctg atatcgaatc aaaaaacaag catggcctca caccactgtt acttggtgta     840
catgagcaaa aacagcaagt cgtgaaattt ttaatcaaga aaaaagcgaa tttaaatgca     900
ctggatagat atggaaggac tgctctcata cttgctgtat gttgtggatc agcaagtata     960
gtcagccttc tacttgagca aaatattgat gtatcttctc aagatctatc tggacagacg    1020
gccagagagt atgctgtttc tagtcatcat catgtaattt gccagttact ttctgactac    1080
aaagaaaaac agatgctaaa aatctcttct gaaaacagca atccagaaaa tgtctcaaga    1140
accagaaata aataa                                                    1155
```

<210> 302
<211> 2000
<212> DNA
<213> Homo sapien

<400> 302

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aggagcaaga tgggcaagtg gtgctgccgt tgcttcccct gctgcaggga gagcggcaag 120
agcaacgtgg gcacttctgg agaccacgac gactctgcta tgaagacact caggagcaag 180
atgggcaagt ggtgccgcca ctgcttcccc tgctgcaggg ggagtggcaa gagcaacgtg 240
ggcgcttctg gagaccacga cgactctgct atgaagacac tcaggaacaa gatgggcaag 300
tggtgctgcc actgcttccc ctgctgcagg gggagcggca agagcaaggt gggcgcttgg 360
ggagactacg atgacagtgc cttcatggag cccaggtacc acgtccgtgg agaagatctg 420
gacaagctcc acagagctgc ctggtggggt aaagtcccca gaaaggatct catcgtcatg 480
ctcagggaca ctgacgtgaa caagaaggac aagcaaaaga ggactgctct acatctggcc 540
tctgccaatg ggaattcaga agtagtaaaa ctcctgctgg acagacgatg tcaacttaat 600
gtccttgaca acaaaaagag gacagctctg ataaaggccg tacaatgcca ggaagatgaa 660
tgtgcgttaa tgttgctgga acatggcact gatccaaata ttccagatga gtatggaaat 720
accactctgc actacgctat ctataatgaa gataaattaa tggccaaagc actgctctta 780
tatggtgctg atatcgaatc aaaaaacaag catggcctca caccactgtt acttggtgta 840
catgagcaaa aacagcaagt cgtgaaattt ttaatcaaga aaaaagcgaa tttaaatgca 900
ctggatagat atggaaggac tgctctcata cttgctgtat gttgtggatc agcaagtata 960
gtcagccttc tacttgagca aaatattgat gtatcttctc aagatctatc tggacagacg 1020
gccagagagt atgctgtttc tagtcatcat catgtaattt gccagttact ttctgactac 1080
aaagaaaaac agatgctaaa aatctcttct gaaaacagca atccagaaca agacttaaag 1140
ctgacatcag aggaagagtc acaaaggttc aaaggcagtg aaaatagcca gccagagaaa 1200
atgtctcaag aaccagaaat aaataaggat ggtgatagag aggttgaaga agaaatgaag 1260
aagcatgaaa gtaataatgt gggattacta gaaaacctga ctaatggtgt cactgctggc 1320
aatggtgata atggattaat tcctcaaagg aagagcagaa cacctgaaaa tcagcaattt 1380
cctgacaacg aaagtgaaga gtatcacaga atttgcgaat tagtttctga ctacaaagaa 1440
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tcagaggaag agtcacaaag gcttgagggc agtgaaaatg gccagccaga gctagaaaat 1560
tttatggcta tcgaagaaat gaagaagcac ggaagtactc atgtcggatt cccagaaaac 1620
ctgactaatg gtgccactgc tggcaatggt gatgatggat taattcctcc aaggaagagc 1680
agaacacctg aaagccagca atttcctgac actgagaatg aagagtatca cagtgacgaa 1740
caaaatgata ctcagaagca attttgtgaa gaacagaaca ctggaatatt acacgatgag 1800
attctgattc atgaagaaaa gcagatagaa gtggttgaaa aaatgaattc tgagctttct 1860
cttagttgta agaaagaaaa agacatcttg catgaaaata gtacgttgcg ggaagaaatt 1920
gccatgctaa gactggagct agacacaatg aaacatcaga gccagctaaa aaaaaaaaaa 1980
aaaaaaaaaa aaaaaaaaaa 2000

<210> 303
<211> 2040
<212> DNA
<213> Homo sapien

<400> 303
atggtggttg aggttgattc catgccggct gcctcttctg tgaagaagcc atttggtctc 60
aggagcaaga tgggcaagtg gtgctgccgt tgcttcccct gctgcaggga gagcggcaag 120
agcaacgtgg gcacttctgg agaccacgac gactctgcta tgaagacact caggagcaag 180
atgggcaagt ggtgccgcca ctgcttcccc tgctgcaggg ggagtggcaa gagcaacgtg 240
ggcgcttctg gagaccacga cgactctgct atgaagacac tcaggaacaa gatgggcaag 300
tggtgctgcc actgcttccc ctgctgcagg gggagcggca agagcaaggt gggcgcttgg 360
ggagactacg atgacagtgc cttcatggag cccaggtacc acgtccgtgg agaagatctg 420
gacaagctcc acagagctgc ctggtggggt aaagtcccca gaaaggatct catcgtcatg 480
ctcagggaca ctgacgtgaa caagaaggac aagcaaaaga ggactgctct acatctggcc 540
tctgccaatg ggaattcaga agtagtaaaa ctcctgctgg acagacgatg tcaacttaat 600

gtccttgaca acaaaaagag gacagctctg ataaaggccg tacaatgcca ggaagatgaa 660
tgtgcgttaa tgttgctgga acatggcact gatccaaata ttccagatga gtatggaaat 720
accactctgc actacgctat ctataatgaa gataaattaa tggccaaagc actgctctta 780
tatggtgctg atatcgaatc aaaaaacaag catggcctca caccactgtt acttggtgta 840
catgagcaaa aacagcaagt cgtgaaattt ttaatcaaga aaaaagcgaa tttaaatgca 900
ctggatagat atggaaggac tgctctcata cttgctgtat gttgtggatc agcaagtata 960
gtcagccttc tacttgagca aaatattgat gtatcttctc aagatctatc tggacagacg 1020
gccagagagt atgctgtttc tagtcatcat catgtaattt gccagttact ttctgactac 1080
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aagcatgaaa gtaataatgt gggattacta gaaaacctga ctaatggtgt cactgctggc 1320
aatggtgata atggattaat tcctcaaagg aagagcagaa cacctgaaaa tcagcaattt 1380
cctgacaacg aaagtgaaga gtatcacaga atttgcgaat tagtttctga ctacaaagaa 1440
aaacagatgc caaaatactc ttctgaaaac agcaacccag aacaagactt aaagctgaca 1500
tcagaggaag agtcacaaag gcttgagggc agtgaaaatg gccagccaga gaaaagatct 1560
caagaaccag aaataaataa ggatggtgat agagagctag aaaattttat ggctatcgaa 1620
gaaatgaaga agcacggaag tactcatgtc ggattcccag aaaacctgac taatggtgcc 1680
actgctggca atggtgatga tggattaatt cctccaagga agagcagaac acctgaaagc 1740
cagcaatttc ctgacactga gaatgaagag tatcacagtg acgaacaaaa tgatactcag 1800
aagcaatttt gtgaagaaca gaacactgga atattacacg atgagattct gattcatgaa 1860
gaaaagcaga tagaagtggt tgaaaaaatg aattctgagc tttctcttag ttgtaagaaa 1920
gaaaaagaca tcttgcatga aaatagtacg ttgcgggaag aaattgccat gctaagactg 1980
gagctagaca caatgaaaca tcagagccag ctaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2040

<210> 304
<211> 384
<212> PRT
<213> Homo sapien

<400> 304
Met Val Val Glu Val Asp Ser Met Pro Ala Ala Ser Ser Val Lys Lys
1 5 10 15
Pro Phe Gly Leu Arg Ser Lys Met Gly Lys Trp Cys Cys Arg Cys Phe
20 25 30
Pro Cys Cys Arg Glu Ser Gly Lys Ser Asn Val Gly Thr Ser Gly Asp
35 40 45
His Asp Asp Ser Ala Met Lys Thr Leu Arg Ser Lys Met Gly Lys Trp
50 55 60
Cys Arg His Cys Phe Pro Cys Cys Arg Gly Ser Gly Lys Ser Asn Val
65 70 75 80
Gly Ala Ser Gly Asp His Asp Asp Ser Ala Met Lys Thr Leu Arg Asn
85 90 95
Lys Met Gly Lys Trp Cys Cys His Cys Phe Pro Cys Cys Arg Gly Ser
100 105 110
Gly Lys Ser Lys Val Gly Ala Trp Gly Asp Tyr Asp Asp Ser Ala Phe
115 120 125
Met Glu Pro Arg Tyr His Val Arg Gly Glu Asp Leu Asp Lys Leu His
130 135 140
Arg Ala Ala Trp Trp Gly Lys Val Pro Arg Lys Asp Leu Ile Val Met
145 150 155 160

```
Leu Arg Asp Thr Asp Val Asn Lys Lys Asp Lys Gln Lys Arg Thr Ala
                165                 170                 175
Leu His Leu Ala Ser Ala Asn Gly Asn Ser Glu Val Val Lys Leu Leu
            180                 185                 190
Leu Asp Arg Arg Cys Gln Leu Asn Val Leu Asp Asn Lys Lys Arg Thr
        195                 200                 205
Ala Leu Ile Lys Ala Val Gln Cys Gln Glu Asp Glu Cys Ala Leu Met
    210                 215                 220
Leu Leu Glu His Gly Thr Asp Pro Asn Ile Pro Asp Glu Tyr Gly Asn
225                 230                 235                 240
Thr Thr Leu His Tyr Ala Ile Tyr Asn Glu Asp Lys Leu Met Ala Lys
                245                 250                 255
Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu Ser Lys Asn Lys His Gly
            260                 265                 270
Leu Thr Pro Leu Leu Leu Gly Val His Glu Gln Lys Gln Gln Val Val
        275                 280                 285
Lys Phe Leu Ile Lys Lys Lys Ala Asn Leu Asn Ala Leu Asp Arg Tyr
    290                 295                 300
Gly Arg Thr Ala Leu Ile Leu Ala Val Cys Cys Gly Ser Ala Ser Ile
305                 310                 315                 320
Val Ser Leu Leu Leu Glu Gln Asn Ile Asp Val Ser Ser Gln Asp Leu
                325                 330                 335
Ser Gly Gln Thr Ala Arg Glu Tyr Ala Val Ser Ser His His His Val
            340                 345                 350
Ile Cys Gln Leu Leu Ser Asp Tyr Lys Glu Lys Gln Met Leu Lys Ile
        355                 360                 365
Ser Ser Glu Asn Ser Asn Pro Glu Asn Val Ser Arg Thr Arg Asn Lys
    370                 375                 380

      <210> 305
      <211> 656
      <212> PRT
      <213> Homo sapien

      <400> 305
Met Val Val Glu Val Asp Ser Met Pro Ala Ala Ser Ser Val Lys Lys
 1               5                  10                  15
Pro Phe Gly Leu Arg Ser Lys Met Gly Lys Trp Cys Cys Arg Cys Phe
            20                  25                  30
Pro Cys Cys Arg Glu Ser Gly Lys Ser Asn Val Gly Thr Ser Gly Asp
        35                  40                  45
His Asp Asp Ser Ala Met Lys Thr Leu Arg Ser Lys Met Gly Lys Trp
    50                  55                  60
Cys Arg His Cys Phe Pro Cys Cys Arg Gly Ser Gly Lys Ser Asn Val
65                  70                  75                  80
Gly Ala Ser Gly Asp His Asp Asp Ser Ala Met Lys Thr Leu Arg Asn
                85                  90                  95
Lys Met Gly Lys Trp Cys Cys His Cys Phe Pro Cys Cys Arg Gly Ser
            100                 105                 110
Gly Lys Ser Lys Val Gly Ala Trp Gly Asp Tyr Asp Asp Ser Ala Phe
        115                 120                 125
```

```
Met Glu Pro Arg Tyr His Val Arg Gly Glu Asp Leu Asp Lys Leu His
    130                 135                 140
Arg Ala Ala Trp Trp Gly Lys Val Pro Arg Lys Asp Leu Ile Val Met
145                 150                 155                 160
Leu Arg Asp Thr Asp Val Asn Lys Lys Asp Lys Gln Lys Arg Thr Ala
                165                 170                 175
Leu His Leu Ala Ser Ala Asn Gly Asn Ser Glu Val Val Lys Leu Leu
            180                 185                 190
Leu Asp Arg Arg Cys Gln Leu Asn Val Leu Asp Asn Lys Lys Arg Thr
        195                 200                 205
Ala Leu Ile Lys Ala Val Gln Cys Gln Glu Asp Glu Cys Ala Leu Met
    210                 215                 220
Leu Leu Glu His Gly Thr Asp Pro Asn Ile Pro Asp Glu Tyr Gly Asn
225                 230                 235                 240
Thr Thr Leu His Tyr Ala Ile Tyr Asn Glu Asp Lys Leu Met Ala Lys
                245                 250                 255
Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu Ser Lys Asn Lys His Gly
            260                 265                 270
Leu Thr Pro Leu Leu Leu Gly Val His Glu Gln Lys Gln Gln Val Val
        275                 280                 285
Lys Phe Leu Ile Lys Lys Lys Ala Asn Leu Asn Ala Leu Asp Arg Tyr
    290                 295                 300
Gly Arg Thr Ala Leu Ile Leu Ala Val Cys Cys Gly Ser Ala Ser Ile
305                 310                 315                 320
Val Ser Leu Leu Leu Glu Gln Asn Ile Asp Val Ser Ser Gln Asp Leu
                325                 330                 335
Ser Gly Gln Thr Ala Arg Glu Tyr Ala Val Ser Ser His His His Val
            340                 345                 350
Ile Cys Gln Leu Leu Ser Asp Tyr Lys Glu Lys Gln Met Leu Lys Ile
        355                 360                 365
Ser Ser Glu Asn Ser Asn Pro Glu Gln Asp Leu Lys Leu Thr Ser Glu
    370                 375                 380
Glu Glu Ser Gln Arg Phe Lys Gly Ser Glu Asn Ser Gln Pro Glu Lys
385                 390                 395                 400
Met Ser Gln Glu Pro Glu Ile Asn Lys Asp Gly Asp Arg Glu Val Glu
                405                 410                 415
Glu Glu Met Lys Lys His Glu Ser Asn Asn Val Gly Leu Leu Glu Asn
            420                 425                 430
Leu Thr Asn Gly Val Thr Ala Gly Asn Gly Asp Asn Gly Leu Ile Pro
        435                 440                 445
Gln Arg Lys Ser Arg Thr Pro Glu Asn Gln Gln Phe Pro Asp Asn Glu
    450                 455                 460
Ser Glu Glu Tyr His Arg Ile Cys Glu Leu Val Ser Asp Tyr Lys Glu
465                 470                 475                 480
Lys Gln Met Pro Lys Tyr Ser Ser Glu Asn Ser Asn Pro Glu Gln Asp
                485                 490                 495
Leu Lys Leu Thr Ser Glu Glu Glu Ser Gln Arg Leu Glu Gly Ser Glu
            500                 505                 510
Asn Gly Gln Pro Glu Leu Glu Asn Phe Met Ala Ile Glu Glu Met Lys
        515                 520                 525
Lys His Gly Ser Thr His Val Gly Phe Pro Glu Asn Leu Thr Asn Gly
```

```
    530                 535                 540
Ala Thr Ala Gly Asn Gly Asp Asp Gly Leu Ile Pro Pro Arg Lys Ser
545                 550                 555                 560
Arg Thr Pro Glu Ser Gln Gln Phe Pro Asp Thr Glu Asn Glu Glu Tyr
                565                 570                 575
His Ser Asp Glu Gln Asn Asp Thr Gln Lys Gln Phe Cys Glu Glu Gln
            580                 585                 590
Asn Thr Gly Ile Leu His Asp Glu Ile Leu Ile His Glu Glu Lys Gln
        595                 600                 605
Ile Glu Val Val Glu Lys Met Asn Ser Glu Leu Ser Leu Ser Cys Lys
    610                 615                 620
Lys Glu Lys Asp Ile Leu His Glu Asn Ser Thr Leu Arg Glu Glu Ile
625                 630                 635                 640
Ala Met Leu Arg Leu Glu Leu Asp Thr Met Lys His Gln Ser Gln Leu
                645                 650                 655
```

<210> 306
<211> 671
<212> PRT
<213> Homo sapien

<400> 306

```
Met Val Val Glu Val Asp Ser Met Pro Ala Ala Ser Ser Val Lys Lys
 1               5                  10                  15
Pro Phe Gly Leu Arg Ser Lys Met Gly Lys Trp Cys Cys Arg Cys Phe
            20                  25                  30
Pro Cys Cys Arg Glu Ser Gly Lys Ser Asn Val Gly Thr Ser Gly Asp
        35                  40                  45
His Asp Asp Ser Ala Met Lys Thr Leu Arg Ser Lys Met Gly Lys Trp
    50                  55                  60
Cys Arg His Cys Phe Pro Cys Cys Arg Gly Ser Gly Lys Ser Asn Val
65                  70                  75                  80
Gly Ala Ser Gly Asp His Asp Asp Ser Ala Met Lys Thr Leu Arg Asn
                85                  90                  95
Lys Met Gly Lys Trp Cys Cys His Cys Phe Pro Cys Cys Arg Gly Ser
            100                 105                 110
Gly Lys Ser Lys Val Gly Ala Trp Gly Asp Tyr Asp Asp Ser Ala Phe
        115                 120                 125
Met Glu Pro Arg Tyr His Val Arg Gly Glu Asp Leu Asp Lys Leu His
    130                 135                 140
Arg Ala Ala Trp Trp Gly Lys Val Pro Arg Lys Asp Leu Ile Val Met
145                 150                 155                 160
Leu Arg Asp Thr Asp Val Asn Lys Lys Asp Lys Gln Lys Arg Thr Ala
                165                 170                 175
Leu His Leu Ala Ser Ala Asn Gly Asn Ser Glu Val Val Lys Leu Leu
            180                 185                 190
Leu Asp Arg Arg Cys Gln Leu Asn Val Leu Asp Asn Lys Lys Arg Thr
        195                 200                 205
Ala Leu Ile Lys Ala Val Gln Cys Gln Glu Asp Glu Cys Ala Leu Met
    210                 215                 220
Leu Leu Glu His Gly Thr Asp Pro Asn Ile Pro Asp Glu Tyr Gly Asn
```

```
225                 230                 235                 240
Thr Thr Leu His Tyr Ala Ile Tyr Asn Glu Asp Lys Leu Met Ala Lys
                245                 250                 255
Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu Ser Lys Asn Lys His Gly
            260                 265                 270
Leu Thr Pro Leu Leu Leu Gly Val His Glu Gln Lys Gln Gln Val Val
        275                 280                 285
Lys Phe Leu Ile Lys Lys Lys Ala Asn Leu Asn Ala Leu Asp Arg Tyr
    290                 295                 300
Gly Arg Thr Ala Leu Ile Leu Ala Val Cys Cys Gly Ser Ala Ser Ile
305                 310                 315                 320
Val Ser Leu Leu Leu Glu Gln Asn Ile Asp Val Ser Ser Gln Asp Leu
                325                 330                 335
Ser Gly Gln Thr Ala Arg Glu Tyr Ala Val Ser Ser His His His Val
            340                 345                 350
Ile Cys Gln Leu Leu Ser Asp Tyr Lys Glu Lys Gln Met Leu Lys Ile
        355                 360                 365
Ser Ser Glu Asn Ser Asn Pro Glu Gln Asp Leu Lys Leu Thr Ser Glu
    370                 375                 380
Glu Glu Ser Gln Arg Phe Lys Gly Ser Glu Asn Ser Gln Pro Glu Lys
385                 390                 395                 400
Met Ser Gln Glu Pro Glu Ile Asn Lys Asp Gly Asp Arg Glu Val Glu
                405                 410                 415
Glu Glu Met Lys Lys His Glu Ser Asn Asn Val Gly Leu Leu Glu Asn
            420                 425                 430
Leu Thr Asn Gly Val Thr Ala Gly Asn Gly Asp Asn Gly Leu Ile Pro
        435                 440                 445
Gln Arg Lys Ser Arg Thr Pro Glu Asn Gln Gln Phe Pro Asp Asn Glu
    450                 455                 460
Ser Glu Glu Tyr His Arg Ile Cys Glu Leu Val Ser Asp Tyr Lys Glu
465                 470                 475                 480
Lys Gln Met Pro Lys Tyr Ser Ser Glu Asn Ser Asn Pro Glu Gln Asp
                485                 490                 495
Leu Lys Leu Thr Ser Glu Glu Glu Ser Gln Arg Leu Glu Gly Ser Glu
            500                 505                 510
Asn Gly Gln Pro Glu Lys Arg Ser Gln Glu Pro Glu Ile Asn Lys Asp
        515                 520                 525
Gly Asp Arg Glu Leu Glu Asn Phe Met Ala Ile Glu Glu Met Lys Lys
    530                 535                 540
His Gly Ser Thr His Val Gly Phe Pro Glu Asn Leu Thr Asn Gly Ala
545                 550                 555                 560
Thr Ala Gly Asn Gly Asp Asp Gly Leu Ile Pro Pro Arg Lys Ser Arg
                565                 570                 575
Thr Pro Glu Ser Gln Gln Phe Pro Asp Thr Glu Asn Glu Glu Tyr His
            580                 585                 590
Ser Asp Glu Gln Asn Asp Thr Gln Lys Gln Phe Cys Glu Glu Gln Asn
        595                 600                 605
Thr Gly Ile Leu His Asp Glu Ile Leu Ile His Glu Glu Lys Gln Ile
    610                 615                 620
Glu Val Val Glu Lys Met Asn Ser Glu Leu Ser Leu Ser Cys Lys Lys
625                 630                 635                 640
```

```
Glu Lys Asp Ile Leu His Glu Asn Ser Thr Leu Arg Glu Glu Ile Ala
                645                 650                 655
Met Leu Arg Leu Glu Leu Asp Thr Met Lys His Gln Ser Gln Leu
            660                 665                 670
```

<210> 307
<211> 800
<212> DNA
<213> Homo sapien

<400> 307

```
atkagcttcc gcttctgaca acactagaga tccctcccct ccctcagggt atggccctcc      60
acttcatttt tggtacataa catctttata ggacaggggt aaaatcccaa tactaacagg     120
agaatgctta ggactctaac aggtttttga gaatgtgttg gtaagggcca ctcaatccaa     180
tttttcttgg tcctccttgt ggtctaggag gacaggcaag ggtgcagatt ttcaagaatg     240
catcagtaag ggccactaaa tccgaccttc ctcgttcctc cttgtggtct gggaggaaaa     300
ctagtgtttc tgttgctgtg tcagtgagca caactattcc gatcagcagg gtccagggac     360
cactgcaggt tcttgggcag ggggagaaac aaaacaaacc aaaaccatgg gcrgttttgt     420
ctttcagatg ggaaacactc aggcatcaac aggctcacct ttgaaatgca tcctaagcca     480
atgggacaaa tttgacccac aaaccctgga aaaagaggtg gctcattttt tttgcactat     540
ggcttggccc caacattctc tctctgatgg ggaaaaatgg ccacctgagg gaagtacaga     600
ttacaatact atcctgcagc ttgacctttt ctgtaagagg gaaggcaaat ggagtgaaat     660
accttatgtc caagctttct tttcattgaa ggagaataca ctatgcaaag cttgaaattt     720
acatcccaca ggaggacctc tcagcttacc cccatatcct agcctcccta tagctccct      780
tcctattagt gataagcctc                                                 800
```

<210> 308
<211> 102
<212> PRT
<213> Homo sapien

<220>
<221> VARIANT
<222> (1)...(102)
<223> Xaa = Any Amino Acid

<400> 308

```
Met Gly Xaa Phe Val Phe Gln Met Gly Asn Thr Gln Ala Ser Thr Gly
 1               5                  10                  15
Ser Pro Leu Lys Cys Ile Leu Ser Gln Trp Asp Lys Phe Asp Pro Gln
            20                  25                  30
Thr Leu Glu Lys Glu Val Ala His Phe Phe Cys Thr Met Ala Trp Pro
        35                  40                  45
Gln His Ser Leu Ser Asp Gly Glu Lys Trp Pro Pro Glu Gly Ser Thr
    50                  55                  60
Asp Tyr Asn Thr Ile Leu Gln Leu Asp Leu Phe Cys Lys Arg Glu Gly
65                  70                  75                  80
Lys Trp Ser Glu Ile Pro Tyr Val Gln Ala Phe Phe Ser Leu Lys Glu
                85                  90                  95
Asn Thr Leu Cys Lys Ala
            100
```

<210> 309
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Made in the lab

<400> 309
Leu Met Ala Glu Glu Tyr Thr Ile Val
1 5

<210> 310
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Made in the lab

<400> 310
Lys Leu Met Ala Lys Ala Leu Leu Leu
1 5

<210> 311
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Made in the lab

<400> 311
Gly Leu Thr Pro Leu Leu Leu Gly Ile
1 5

<210> 312
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Made in the lab

<400> 312
Lys Leu Val Leu Asp Arg Arg Cys Gln Leu
1 5 10

<210> 313
<211> 1852

<212> DNA
<213> Homo sapiens

<400> 313
ggcacgagaa ttaaaaccct cagcaaaaca ggcatagaag ggacatacct taaagtaata 60
aaaaccacct atgacaagcc cacagccaac ataatactaa atggggaaaa gttagaagca 120
tttcctctga gaactgcaac aataaataca aggatgctgg attttgtcaa atgccttttc 180
tgtgtctgtt gagatgctta tgtgactttg cttttaattc tgtttatgtg attatcacat 240
ttattgactt gcctgtgtta gaccggaaga gctggggtgt ttctcaggag ccaccgtgtg 300
ctgcggcagc ttcgggataa cttgaggctg catcactggg gaagaaacac aytcctgtcc 360
gtggcgctga tggctgagga cagagcttca gtgtggcttc tctgcgactg gcttcttcgg 420
ggagttcttc cttcatagtt catccatatg gctccagagg aaaattatat tattttgtta 480
tggatgaaga gtattacgtt gtgcagatat actgcagtgt cttcatctct tgatgtgtga 540
ttgggtaggt tccaccatgt tgccgcagat gacatgattt cagtacctgt gtctggctga 600
aaagtgtttg tttgtgaatg gatattgtgg tttctggatc tcatcctctg tgggtggaca 660
gctttctcca ccttgctgga agtgacctgc tgtccagaag tttgatggct gaggagtata 720
ccatcgtgca tgcatctttc atttcctgca tttcttcctc cctggatgga caggggggagc 780
ggcaagagca acgtgggcac ttctggagac cacaacgact cctctgtgaa gacgcttggg 840
agcaagaggt gcaagtggtg ctgccactgc ttcccctgct gcaggggggag cggcaagagc 900
aacgtggtcg cttggggaga ctacgatgac agcgccttca tggatcccag gtaccacgtc 960
catggagaag atctggacaa gctccacaga gctgcctggt ggggtaaagt ccccagaaag 1020
gatctcatcg tcatgctcag ggacacggat gtgaacaaga gggacaagca aaagaggact 1080
gctctacatc tggcctctgc caatgggaat tcagaagtag taaaactcgt gctggacaga 1140
cgatgtcaac ttaatgtcct tgacaacaaa aagaggacag ctctgacaaa ggccgtacaa 1200
tgccaggaag atgaatgtgc gttaatgttg ctggaacatg gcactgatcc aaatattcca 1260
gatgagtatg gaaataccac tctacactat gctgtctaca atgaagataa attaatggcc 1320
aaagcactgc tcttatacgg tgctgatatc gaatcaaaaa acaagcatgg cctcacacca 1380
ctgctacttg gtatacatga gcaaaaacag caagtggtga aatttttaat caagaaaaaa 1440
gcgaatttaa atgcgctgga tagatatgga agaactgctc tcatacttgc tgtatgttgt 1500
ggatcagcaa gtatagtcag ccctctactt gagcaaaatg ttgatgtatc ttctcaagat 1560
ctggaaagac ggccagagag tatgctgttt ctagtcatca tcatgtaatt tgccagttac 1620
tttctgacta caaagaaaaa cagatgttaa aaatctcttc tgaaaacagc aatccagaac 1680
aagacttaaa gctgacatca gaggaagagt cacaaaggct taaaggaagt gaaaacagcc 1740
agccagagct agaagattta tggctattga agaagaatga agaacacgga agtactcatg 1800
tgggattccc agaaaacctg actaacggtg ccgctgctgg caatggtgat ga 1852

<210> 314
<211> 879
<212> DNA
<213> Homo sapiens

<400> 314
atgcatcttt catttcctgc atttcttcct ccctggatgg acaggggggag cggcaagagc 60
aacgtgggca cttctggaga ccacaacgac tcctctgtga agacgcttgg gagcaagagg 120
tgcaagtggt gctgccactg cttcccctgc tgcaggggga gcggcaagag caacgtggtc 180
gcttggggag actacgatga cagcgccttc atggatccca ggtaccacgt ccatggagaa 240
gatctggaca agctccacag agctgcctgg tggggtaaag tccccagaaa ggatctcatc 300
gtcatgctca gggacacgga tgtgaacaag agggacaagc aaaagaggac tgctctacat 360
ctggcctctg ccaatgggaa ttcagaagta gtaaaactcg tgctggacag acgatgtcaa 420
cttaatgtcc ttgacaacaa aaagaggaca gctctgacaa aggccgtaca atgccaggaa 480
gatgaatgtg cgttaatgtt gctggaacat ggcactgatc caaatattcc agatgagtat 540

ggaaatacca ctctacacta tgctgtctac aatgaagata aattaatggc caaagcactg 600
ctcttatacg gtgctgatat cgaatcaaaa aacaagcatg gcctcacacc actgctactt 660
ggtatacatg agcaaaaaca gcaagtggtg aaatttttaa tcaagaaaaa agcgaattta 720
aatgcgctgg atagatatgg aagaactgct ctcatacttg ctgtatgttg tggatcagca 780
agtatagtca gccctctact tgagcaaaat gttgatgtat cttctcaaga tctggaaaga 840
cggccagaga gtatgctgtt tctagtcatc atcatgtaa 879

<210> 315
<211> 293
<212> PRT
<213> Homo sapiens

<400> 315
Met His Leu Ser Phe Pro Ala Phe Leu Pro Pro Trp Met Asp Arg Gly
5 10 15

Ser Gly Lys Ser Asn Val Gly Thr Ser Gly Asp His Asn Asp Ser Ser
20 25 30

Val Lys Thr Leu Gly Ser Lys Arg Cys Lys Trp Cys Cys His Cys Phe
35 40 45

Pro Cys Cys Arg Gly Ser Gly Lys Ser Asn Val Val Ala Trp Gly Asp
50 55 60

Tyr Asp Asp Ser Ala Phe Met Asp Pro Arg Tyr His Val His Gly Glu
65 70 75 80

Asp Leu Asp Lys Leu His Arg Ala Ala Trp Trp Gly Lys Val Pro Arg
85 90 95

Lys Asp Leu Ile Val Met Leu Arg Asp Thr Asp Val Asn Lys Arg Asp
100 105 110

Lys Gln Lys Arg Thr Ala Leu His Leu Ala Ser Ala Asn Gly Asn Ser
115 120 125

Glu Val Val Lys Leu Val Leu Asp Arg Arg Cys Gln Leu Asn Val Leu
130 135 140

Asp Asn Lys Lys Arg Thr Ala Leu Thr Lys Ala Val Gln Cys Gln Glu
145 150 155 160

Asp Glu Cys Ala Leu Met Leu Leu Glu His Gly Thr Asp Pro Asn Ile
165 170 175

Pro Asp Glu Tyr Gly Asn Thr Thr Leu His Tyr Ala Val Tyr Asn Glu
180 185 190

Asp Lys Leu Met Ala Lys Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu
195 200 205

Ser Lys Asn Lys His Gly Leu Thr Pro Leu Leu Leu Gly Ile His Glu
210 215 220

Gln Lys Gln Gln Val Val Lys Phe Leu Ile Lys Lys Lys Ala Asn Leu
225 230 235 240

Asn Ala Leu Asp Arg Tyr Gly Arg Thr Ala Leu Ile Leu Ala Val Cys
245 250 255

Cys Gly Ser Ala Ser Ile Val Ser Pro Leu Leu Glu Gln Asn Val Asp
260 265 270

Val Ser Ser Gln Asp Leu Glu Arg Arg Pro Glu Ser Met Leu Phe Leu
275 280 285

Val Ile Ile Met
290

<210> 316
<211> 584
<212> DNA
<213> Homo sapiens

<400> 316
agttgggcca aattcccctc cccctacagc ttgaagggga cataaccaat agcctggggt 60
ttttttgtgg tcctttggag atttctttgc ttatttttctt ctgggtgggg gtgattagag 120
gaggcttatc actaatagga aggggagcta tagggaggct aggatatggg ggtaagctga 180
gaggtcctcc tgtgggatgt aaatttcaag ctttgcatag tgtattctcc ttcaatgaaa 240
agaaagcttg gacataaggt atttcactcc atttgccttc cctcttacag aaaaggtcaa 300
gctgcaggat agtattgtaa tctgtacttc cctcaggtgg ccatttttcc ccatcagaga 360
gagaatgttg gggccaagcc atagtgcaga aaaaaaaatg agccacctct ttttccaggg 420
tttgtgggtc aaatttgtcc cattggctta ggatgcattt caaaggtgag cctgttgatg 480
cctgagtgtt tcccatctga aagacaaaac tgcccatggt tttggtttgt tttgtttctc 540
cccctgccca agaactatca aactcctgag ccaacaacta aaaa 584

<210> 317
<211> 829
<212> DNA
<213> Homo sapiens

<400> 317
attagcttcc gcttctgaca acactagaga tccctccccct ccctcagggt atggccctcc 60
acttcatttt tggtacataa catctttata ggacaggggt aaaatcccaa tactaacagg 120
agaatgctta ggactctaac aggtttttga gaatgtgttg gtaagggcca ctcaatccaa 180
tttttcttgg tcctccttgt ggtctaggag gacaggcaag ggtgcagatt ttcaagaatg 240
catcagtaag ggccactaaa tccgaccttc ctcgttcctc cttgtggtct gggaggaaaa 300
ctagtgtttc tgttgctgtg tcagtgagca caactattcc gatcagcagg gtccagggac 360
cactgcaggt tcttgggcag ggggagaaac aaaacaaacc aaaaccatgg gcagttttgt 420
ctttcagatg ggaaacactc aggcatcaac aggctcacct ttgaaatgca tcctaagcca 480
atgggacaaa tttgacccac aaaccctgga aaaagaggtg gctcattttt tttgcactat 540

ggcttggccc caacattctc tctctgatgg ggaaaaatgg ccacctgagg gaagtacaga 600
ttacaatact atcctgcagc ttgacctttt ctgtaagagg gaaggcaaat ggagtgaaat 660
accttatgtc caagctttct tttcattgaa ggagaataca ctatgcaaag cttgaaattt 720
acatcccaca ggaggacctc tcagcttacc cccatatcct agcctcccta tagctcccct 780
tcctattagt gataagcctc ctctaatcac ccccacccag aagaaaata 829

<210> 318
<211> 30
<212> PRT
<213> Homo sapien

<400> 318
Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe
1 5 10 15

Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile
20 25 30

<210> 319
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 319

ggcctctgcc aatgggaact cagaagtagt aaaactcctg c 41

<210> 320
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 320

gcaggagttt tactacttct gagttcccat tggcagaggc c 41

<210> 321
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 321

ggggaattcc cgctggtgcc gcgcggcagc cctatggtgg ttgaggttga 50
ttccatgccg 60

<210> 322
<211> 42
<212> DNA
<213> Artificial Sequnce

<220>
<223> PCR primer

<400> 322

cccgaattct tatttatttc tggttcttga gacattttct gg 42

<210> 323
<211> 1590
<212> DNA
<213> Homo sapiens

<400> 323
atgcatcacc atcaccatca cacggccgcg tccgataact tccagctgtc ccagggtggg 60
cagggattcg ccattccgat cgggcaggcg atggcgatcg cgggccagat caagcttccc 120
accgttcata tcgggcctac cgccttcctc ggcttgggtg ttgtcgacaa caacggcaac 180
ggcgcacgag tccaacgcgt ggtcgggagc gctccggcgg caagtctcgg catctccacc 240
ggcgacgtga tcaccgcggt cgacggcgct ccgatcaact cggccaccgc gatggcggac 300
gcgcttaacg ggcatcatcc cggtgacgtc atctcggtga cctggcaaac caagtcgggc 360
ggcacgcgta cagggaacgt gacattggcc gagggacccc cggccgaatt cccgctggtg 420
ccgcgcggca gccctatggt ggttgaggtt gattccatgc cggctgcttc ttctgtgaag 480
aagccatttg gtctcaggag caagatgggc aagtggtgct gccgttgctt cccctgctgc 540
agggagagcg gcaagagcaa cgtgggcact tctggagacc acgacgactc tgctatgaag 600
acactcagga gcaagatggg caagtggtgc cgccactgct tcccctgctg caggggagt 660
ggcaagagca acgtgggcgc ttctggagac cacgacgact ctgctatgaa gacactcagg 720
aacaagatgg gcaagtggtg ctgccactgc ttcccctgct gcaggggggag cggcaagagc 780
aaggtgggcg cttggggaga ctacgatgac agygccttca tggagcccag gtaccacgtc 840
cgtggagaag atctggacaa gctccacaga gctgcctggt ggggtaaagt ccccagaaag 900
gatctcatcg tcatgctcag ggacactgac gtgaacaaga aggacaagca aaagaggact 960
gctctacatc tggcctctgc caatgggaat tcagaagtag taaaactcct gctggacaga 1020
cgatgtcaac ttaatgtcct tgacaacaaa aagaggacag ctctgataaa ggccgtacaa 1080
tgccaggaag atgaatgtgc gttaatgttg ctggaacatg gcactgatcc aaatattcca 1140
gatgagtatg gaaataccac tctgcactac gctatctata atgaagataa attaatggcc 1200
aaagcactgc tcttatatgg tgctgatatc gaatcaaaaa acaagcatgg cctcacacca 1260
ctgttacttg gtgtacatga gcaaaaacag caagtcgtga aatttttaat caagaaaaaa 1320
gcgaatttaa atgcactgga tagatatgga aggactgctc tcatacttgc tgtatgttgt 1380
ggatcagcaa gtatagtcag ccttctactt gagcaaaata ttgatgtatc ttctcaagat 1440

ctatctggac agacggccag agagtatgct gtttctagtc atcatcatgt aatttgccag 1500
ttactttctg actacaaaga aaaacagatg ctaaaaatct cttctgaaaa cagcaatcca 1560
gaaaatgtct caagaaccag aaataaataa 1590

<210> 324
<211> 529
<212> PRT
<213> Homo sapiens

<400> 324
Met His His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
1 5 10 15

Ser Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
20 25 30

Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala
35 40 45

Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
50 55 60

Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
65 70 75 80

Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
85 90 95

Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
100 105 110

Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
115 120 125

Leu Ala Glu Gly Pro Pro Ala Glu Phe Pro Leu Val Pro Arg Gly Ser
130 135 140

Pro Met Val Val Glu Val Asp Ser Met Pro Ala Ala Ser Ser Val Lys
145 150 155 160

Lys Pro Phe Gly Leu Arg Ser Lys Met Gly Lys Trp Cys Cys Arg Cys
165 170 175

Phe Pro Cys Cys Arg Glu Ser Gly Lys Ser Asn Val Gly Thr Ser Gly
180 185 190

Asp His Asp Asp Ser Ala Met Lys Thr Leu Arg Ser Lys Met Gly Lys
195 200 205

Trp Cys Arg His Cys Phe Pro Cys Cys Arg Gly Ser Gly Lys Ser Asn

210 215 220

Val Gly Ala Ser Gly Asp His Asp Asp Ser Ala Met Lys Thr Leu Arg
225 230 235 240

Asn Lys Met Gly Lys Trp Cys Cys His Cys Phe Pro Cys Cys Arg Gly
245 250 255

Ser Gly Lys Ser Lys Val Gly Ala Trp Gly Asp Tyr Asp Asp Ser Ala
260 265 270

Phe Met Glu Pro Arg Tyr His Val Arg Gly Glu Asp Leu Asp Lys Leu
275 280 285

His Arg Ala Ala Trp Trp Gly Lys Val Pro Arg Lys Asp Leu Ile Val
290 295 300

Met Leu Arg Asp Thr Asp Val Asn Lys Lys Asp Lys Gln Lys Arg Thr
305 310 315 320

Ala Leu His Leu Ala Ser Ala Asn Gly Asn Ser Glu Val Val Lys Leu
325 330 335

Leu Leu Asp Arg Arg Cys Gln Leu Asn Val Leu Asp Asn Lys Lys Arg
340 345 350

Thr Ala Leu Ile Lys Ala Val Gln Cys Gln Glu Asp Glu Cys Ala Leu
355 360 365

Met Leu Leu Glu His Gly Thr Asp Pro Asn Ile Pro Asp Glu Tyr Gly
370 375 380

Asn Thr Thr Leu His Tyr Ala Ile Tyr Asn Glu Asp Lys Leu Met Ala
385 390 395 400

Lys Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu Ser Lys Asn Lys His
405 410 415

Gly Leu Thr Pro Leu Leu Leu Gly Val His Glu Gln Lys Gln Gln Val
420 425 430

Val Lys Phe Leu Ile Lys Lys Lys Ala Asn Leu Asn Ala Leu Asp Arg
435 440 445

Tyr Gly Arg Thr Ala Leu Ile Leu Ala Val Cys Cys Gly Ser Ala Ser
450 455 460

Ile Val Ser Leu Leu Leu Glu Gln Asn Ile Asp Val Ser Ser Gln Asp
465 470 475 480

Leu Ser Gly Gln Thr Ala Arg Glu Tyr Ala Val Ser Ser His His His

485 490 495

Val Ile Cys Gln Leu Leu Ser Asp Tyr Lys Glu Lys Gln Met Leu Lys
500 505 510

Ile Ser Ser Glu Asn Ser Asn Pro Glu Asn Val Ser Arg Thr Arg Asn
515 520 525

Lys